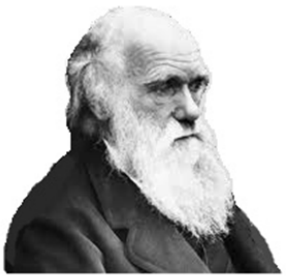
Available online at www.worldnewsnaturalsciences.com



World News of Natural Sciences

An International Scientific Journal

WNOFNS 49 (2023) 137-146 EISSN 2543-5426

Bacteriological Evaluation of Hydrocarbon Polluted Soil in Obitti Oil Field, Ohaji-Egbema, Imo State, Nigeria

M. C. Maduwuba[1,*] and **J. O. Akwarandu**[2]

[1] Department of Microbiology, Faculty of Biological Sciences, Imo State University, Omuma, Imo State Nigeria

[2] Department of Science Laboratory Technology, Imo State Polytechnic, Omuma, Imo State Nigeria

*E-mail address :mjaychichi1@yahoo.com

ABSTRACT

The bacteriological evaluation of hydrocarbon polluted soil in Obitti oil field in Ohaji Egbema was investigated to ascertain the presence of bacterial community in crude oil-polluted soil. Ohaji Egbema has experienced crude oil pollution in recent times due to the presence of crude oil exploration facilities including the Obitti oil field. Soil samples were collected from four (4) different points within the oil field with varying degree of crude oil pollution along with their corresponding control samples (unpolluted soil). The total culturable heterotrophic bacteria (TCHB), total culturable hydrocarbon utilizing bacteria (TCHUB), pH and total petroleum hydrocarbon (TPH) concentrations were monitored using standard procedures. The total culturable heterotrophic bacterial count ranged from $0.45\pm2.0x10^5$ Cfu/g to $2.12\pm2.6x10^5$ Cfu/g while total culturable hydrocarbon utilizing bacterial count ranged from $0.56\pm2.1x10^4$ Cfu/g to $1.35\pm2.6x10^3$ Cfu/g. pH ranged from 0.11±4.0 to 1.10±5.0 for the polluted soil samples while a range of 0.10±5.7 to 1.00±7.2 was recorded for the control samples. The TPH analysis revealed a high concentration of 9.51±5747.13 mg/kg to 3.45±7214.82 mg/kg for polluted soil samples which is above the DPR intervention limit of 5000 mg/kg for soils while the control samples recorded a range of 5.41±3118.29 mg/kg to 8.21±4285.02 mg/kg. This study has therefore revealed the ability of indigenous bacterial population to strive despite crude oil pollution and as such these hydrocarbon impacted sites can be harnessed for the isolation of hydrocarbonoclastic bacteria which can serve as a bio-resource for the effective bioremediation of hydrocarbon impacted environment.

***Keywords*:** Crude oil, Soil, Hydrocarbon, Bacteria, Pollution, Degradation

(Received 02 May 2023; Accepted 26 May 2023; Date of Publication 27 May 2023)

1. INTRODUCTION

In recent years, a lot of oil-producing locations have been experiencing the consequences of oil spillage of which Ohaji Egbema is not left out. According to Barberan *et al.*[1], approximately five million tons of crude and refined oil are lost to the environment every year as a result of anthropogenic sources such as oil spill. Oil-spill pollution is hazardous to man and the environment [2].

Contamination always occurs in the course of oil exploration activities, oil transportation, storage and usage of petroleum products and accidental spills [1,2]. The burden of oil spill has created the consciousness in lots of people who have now become informed of the need of protecting the ecosystem and also estimate the level of damage caused by the contamination. Crude oil pollution can also cause mutation, cancer and even death to other organisms inhabiting such ecosystem [3].

Crude oil derived hydrocarbon accumulation in the environment is considered a xenobiotic phenomenon capable of enhancing microbial metabolism and stimulating the proliferation of hydrocarbon-degrading microorganisms [4]. Microbial community diversity is affected by a number of environmental factors, including the degree of pollution, duration of exposure and type of pollutant.

High concentrations of the contaminants can select or favor specific microbial taxa and reduce microbial biomass, either by inhibiting sensitive microbes or by promoting the growth of tolerant or biodegrading bacteria. The changes in bacterial community diversity can significantly influence ecosystem structure and function, biodegradation potential and environmental health [5]. The stress impacted by crude oil pollution on microorganisms may lead to a significant increase in enzyme expression associated with the metabolism of BTEX and polycyclic aromatic hydrocarbons. This response is an adaptation strategy used by microbial communities in the environment [6].

The occurrence and dangers of oil pollution has also led to extensive research on spill management. The environmental changes resulting from the petroleum spill have currently aroused the concern of chemists, environmentalists, engineers and biotechnologists [7,8]. Enormous resources have been spent by the Nigeria government on the cleaning and management of petroleum spills. Lives have also been lost due to the health hazards associated with oil spills [9]. Aeration of soil where oil spill occur is reduced leading to low yield of agricultural produce as aquatic lives are not left out. The bacterial cell numbers are also affected leading to reduction in the activities of certain nutrient cycling enzymes. Contaminants can impact microbial dynamics, leading to changes in the community composition, including direct toxicity or competition [10].

The presence of petroleum derived hydrocarbons in soils affects the soil moisture content, nutrient availability and content, aeration, pH and microbial composition [11].

The disappearance of spilled crude from the environment is attributable to the activities of the microflora of the soil. The ability to utilize hydrocarbon substrates is demonstrated by a variety of bacterial genera which are widely distributed in both oil-polluted and pristine environment [12,13].

A lot of microbial ecologists have isolated various bacteria species that can effectively degrade hydrocarbons in the natural environment. Most of these bacteria species were isolated from heavily contaminated coastal areas based on their ability to metabolize different carbon sources such as aliphatic and aromatic compounds and their chlorinated derivatives [14].

In a study conducted by Mahjoubi *et al*.[15] on bacteria contamination of hydrocarbons, *Pseudomonas, Ochrabactrum, Bacillus, Agrobacterium, Stenotrophomonas, Brevundimonas, Gordonia, Acinetobacter* etc species were isolated as the possible contaminants.

Evaluating the bacterial genera associated with hydrocarbon polluted soils helps to provide valuable clues for environmental restoration and management [2]. The distribution of microorganisms especially bacteria is an important factor in natural attenuation and biological degradation pattern by indigenous microbial flora.

Ohaji Egbema is the major oil producing community in Imo State Nigeria. This area has experienced incidences of oil pollution over the years as a result of increasing oil exploration activities in the community. This has drastically affected the quality of life in this area. It has also affected crop yield since this area is also known for agriculture. It has also affected the aquatic ecosystem in this area thus negatively impacting on aquatic organisms. Hence, this study is aimed at evaluating the bacteriological quality of the petroleum derived hydrocarbon contaminated site in Obitti community Ohaji-Egbema L.G.A Imo State Nigeria.

2. MATERIALS AND METHODS

Study Site Description

Samples were collected from crude oil polluted oil field in Obitti Community Ohaji-Egbema Imo State, Nigeria. This area has experienced crude oil pollution from oil exploration activities by oil companies. The co-ordinates of the sample sites as evaluated using the Global Positioning System (GPS) are; N 050 321 5811 E 060 941 8511.

Sample Collection

Five soil samples were collected aseptically from different points in the oil field using soil auger at the depth of 0 – 30 cm. Each of the soil sample was homogenized and put into a sterile black polyethylene bag. Control sample was collected 200 m away from the polluted site where there was no oil contamination. The samples were labeled appropriately (S1-S6). All samples were then transported to the laboratory at 40 °C in an ice chest [16].

Enumeration of Total Culturable Bacterial Population

The total culturable heterotrophic bacterial count was done using the nutrient agar (Accumedia, Sweden). The medium was prepared following the manufacturer's instructions. Serial dilution was done according to the method of APHA [17]. A volume of 100 µL each of 10-4 – 10-7 dilutions of the individual samples were spread on the nutrient agar in duplicates. The inoculated plates were incubated at 30 °C for 24 h.

Similarly, counts for total hydrocarbon utilizing bacteria was carried out using the Bushnell Haas Agar (with 1 % v/v onshore crude oil) amended with 0.01 % w/v nystatin according to the method of Hamamura *et al.* [18]. All total viable cells were estimated in Cfu/g.

Physico-chemical Analysis of Samples

The pH of the individual samples was determined using the method of Bates [19] with the aid of a pH meter (AI-552).

Total Petroleum Hydrocarbon (TPH) Analysis

This was determined by the gas chromatography technique using the gas chromatography-flame ionization detector (GC-FID) system HP5892 series II, USA according to the method of Maduwuba [20] and Akpoveta *et al.* [21].

3. RESULTS

The total culturable heterotrophic bacterial count (TCHBC) is presented in Figure 1. Sample A recorded the highest heterotrophic bacterial count of 2.12 ± 2.6 × 105 cfulg followed by sample B with 1.11 ± 2.5 × 105 cfulg then sample D 0.77 ± 2.3 × 105 cfulg while sample C had the lowest heterotrophic bacterial count of 0.45 ± 2.0 × 105 cfulg. For their controls, control B recorded the highest heterotrophic bacterial count of 1.21 ± 2.1 106 cfulg while control C had the lowest count of 1.20 ± 1.3 × 106 cfulg. The controls recorded higher heterotrophic counts compared to the polluted soil samples.

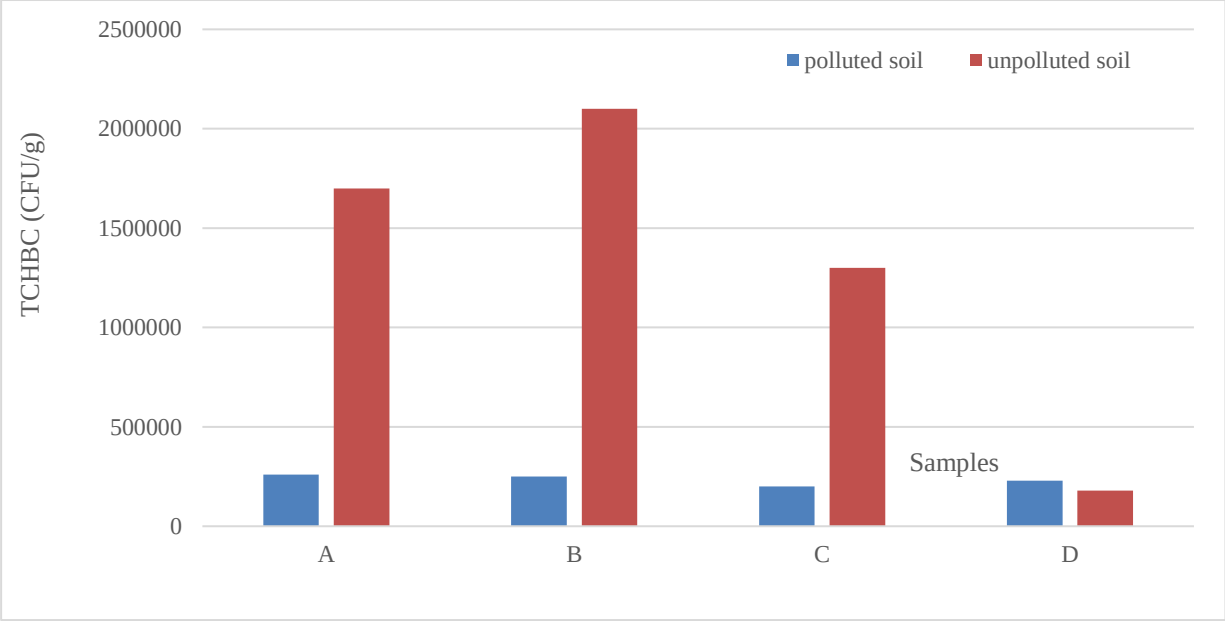


Figure 1. Total culturable heterotrophic bacterial count of polluted and unpolluted soils

The total culturable hydrocarbon utilizing bacterial count (TCHUBC) is presented in Figure 2. Sample C recorded the highest count of 0.56 ± 2.1 × 104 cfulg while its control had 1.21 ± 0.9 × 103 cfulg. This is followed by sample A with 0.43 ± 2.0 × 104 cfulg and 1.0 ± 1.0 × 103 cfulg for control A then sample D with 2.00 ± 1.7 × 104 cfulg and 0.92 ± 1.1 × 103 cfulg for control D. Sample B had 1.35 ± 2.6 × 103 cfulg with control B recording 1.12 ± 0.7 × 103 cfulg. The polluted soil samples recorded higher hydrocarbon utilizing bacterial counts compared to the control (unpolluted) samples.

The pH values of all samples with their corresponding control is presented in Figure 3. The results of the analysis reveals that sample B had highest pH value of 1.10 ± 5.0 and control

B 1.00 ± 7.2, followed by sample C which had pH value of 0.22 ± 4.8 and control C 0.23 ± 6.2. The pH values of 0.21 ± 4.2 and 0.12 ± 6.0 were recorded by sample D and control D respectively while sample A and control A recorded 0.11 ± 4.0 and 0.10 ± 5.7 accordingly.

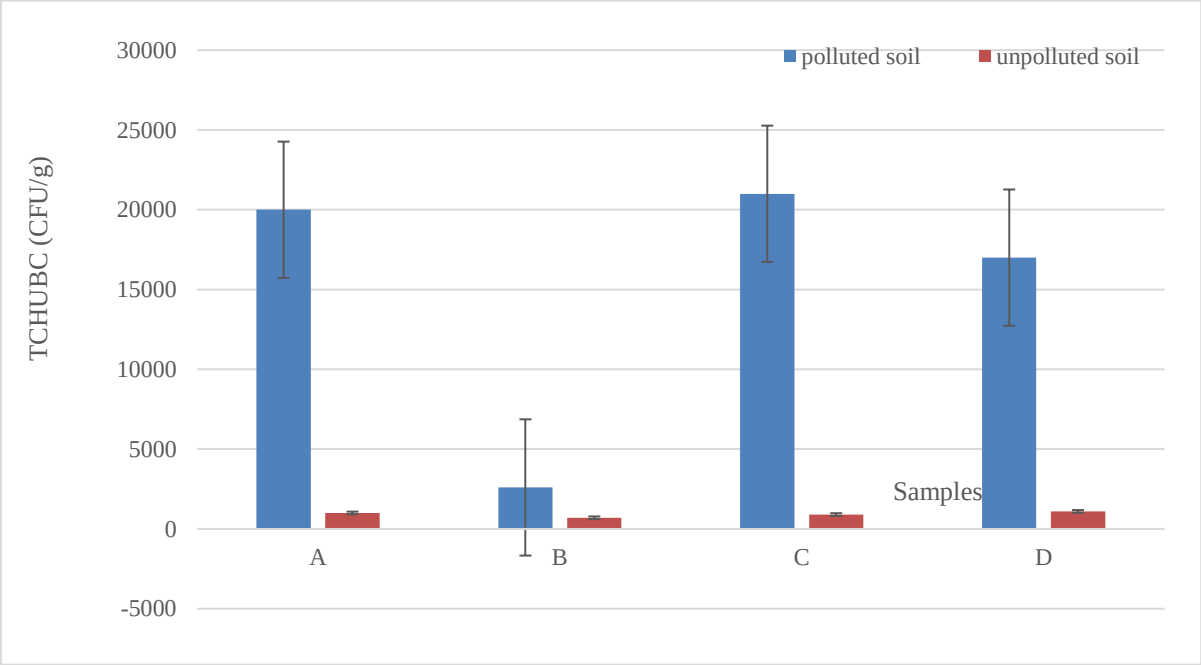


Figure 2. Total culturable hydrocarbon utilizing bacterial count of polluted and unpolluted soils

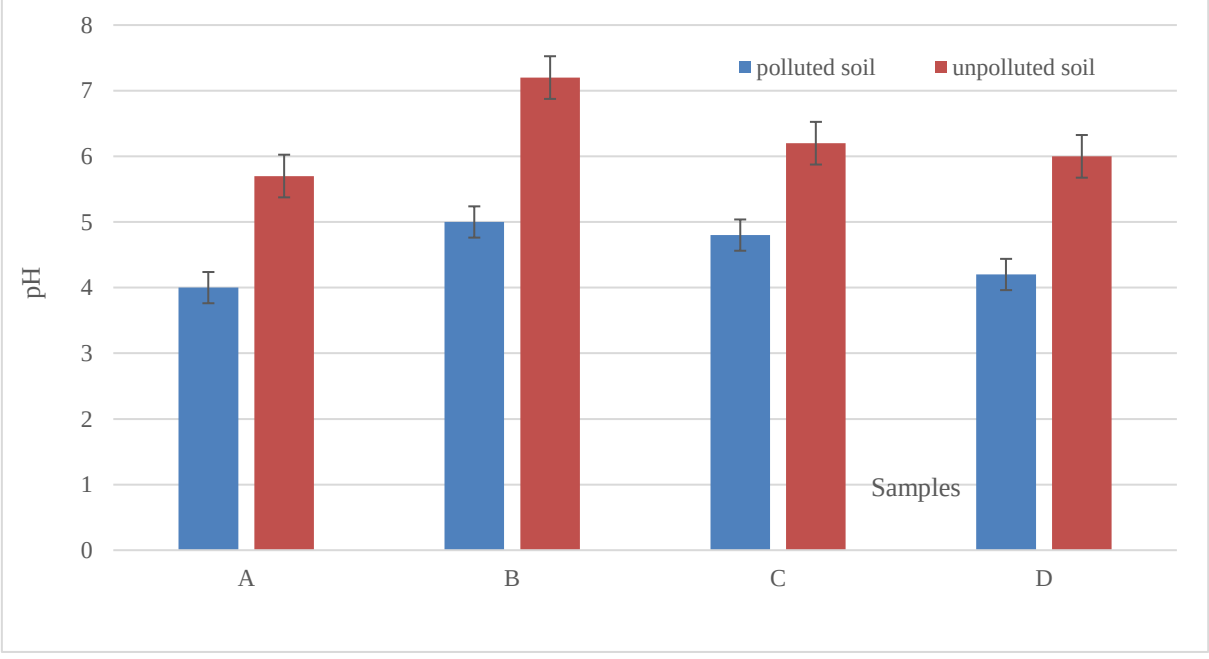


Figure 3. pH of polluted and unpolluted soil

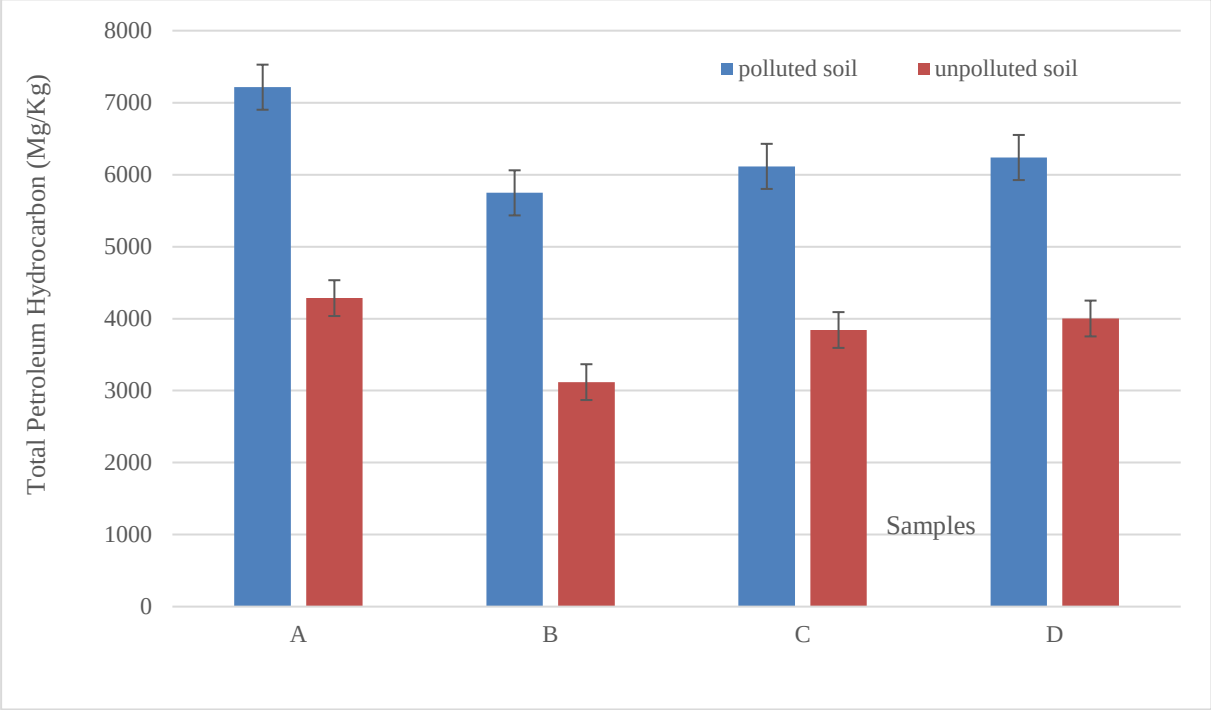


Figure 4. Total petroleum hydrocarbon of polluted and unpolluted soils.

The gas chromatograpy (GC) analysis revealed that sample A recorded the highest TPH concentration of 3.45 ± 7214.82 mg/kg while control A was 8.21 ± 4285.02 mg/kg. This was followed by sample D with a concentration of 7.34 ± 6238.27 mg/kg while control D was 6.19 ± 4002.10 mg/kg. Sample C recorded a TPH concentration of 4.55 ± 6114.96 mg/kg with control C having 5.55 ± 3842.18 mg/kg while sample B had the lowest TPH concentration of 9.51 ± 5747.13 mg/kg and 5.41 ± 3118.29 mg/kg for control B. The TPH concentrations of the individual samples and there corresponding control is represented in Figure 4. The TPH concentrations of the polluted soil samples were higher than that of the control samples.

4. DISCUSSION

The result of the total culturable heterotrophic bacterial count (TCHBC) as shown in Figure 1 is an indication that the resident bacterial community present in the soil were able to proliferate despite changes in chemical composition due to crude oil pollution of the soil environment. This is in-line with the study conducted by Leera *et al*. [22] which revealed that some indigenous microbial flora can still strive in a soil polluted with hydrocarbon. When compared to the unpolluted soil (control samples), it was evident that the resident bacterial community population was affected since the bacterial population of the unpolluted soils were higher than that of the polluted soils. The result also confirms the study conducted by Chikere and Ekwuabu [23] that bacterial community can still withstand and survive toxic crude oil concentrations. The stability of the bacterial population isolated from this site points to the adaptation and survival ability of the bacterial community present in this environment despite the presence of crude oil pollution [24].

The total culturable hydrocarbon utilizing bacterial count (TCHUBC) presented in figure 2 also shows the ability of indigenous hydrocarbon utilizing bacterial population to adapt and utilize the crude oil as their carbon and energy source leading to their proliferation. A similar observation was reported by Eze and Okpokwasili [25] and Maduwuba, *et al* [16] where they reported higher counts of hydrocarbon utilizing bacteria in a crude oil polluted soil compared to its control samples which were unpolluted. The difference in the TCHUBC of the different samples could be attributed to the variation in the degree of crude oil pollution of the different points sampled. The variation in physicochemical characteristics such as pH, organic carbon content, redox potential, moisture content, phosphorus and nitrogen content could also be a contributing factor [26,27]. Also, the duration of pollution and type of pollutant is another vital factor that can influence the proliferation of hydrocarbon-utilizing bacteria [28].

The pH analysis revealed that samples A and D were highly acidic while samples B and C were moderately acidic. The results of the unpolluted soil samples showed slight acidity to neutral pH indicating that the variation in pH could have resulted from the crude oil pollution as reported by Yi *et al*. [29] in a similar study. According to Ogbo and Okhuoya [30], the variation in pH values could have also been as a result of the by-products of bacterial metabolism and the presence of other organic compounds in the soil. The acidic state of the polluted soils can affect the microbial population thus causing an ecological shift. The acidic pH of the polluted soil may also influence the rate of bacterial growth, the bacterial population dynamics and the bacterial genera present in the soil. This can impair plant growth and other ecosystem function [31].

The TPH monitoring revealed the degree of hydrocarbon pollution in the soil samples. The polluted soil samples exceeded the DPR intervention limit of 5000 mg/kg for TPH in soil indicating high degree of hydrocarbon pollution from crude oil while the unpolluted samples were below the DPR intervention limits for TPH. This high TPH concentration is detrimental to the environment as it could affect soil fertility, productivity, microbial composition and diversity [32]. The hydrocarbons could also be carried through leaching and run-offs into the aquatic environment and the water aquifer [33]. The TPH analysis also revealed the presence of low to high molecular weight hydrocarbons. These hydrocarbons possess strong molecular bonds, making them persistence in the environment. Also, their hydrophobic characteristics and low solubility in water makes them recalcitrant thus taking a longer time to degrade [34].

The presence of these hydrocarbons not only affect the bacterial community diversity and ecosystem structure and function of this environment, some concentrations could be leached into the soil aquifer which will pose a threat to human lives. Also some of the hydrocarbons can be deposited into nearby streams and river affecting fishes and other aquatic lives in that environment [35].

5. CONCLUSION

The increase in crude oil exploration activities in Obitti community Ohaji Egbema LGA needs to be given urgent attention because it has caused several incidence of pollution and oil fires in recently. This has also affected the organisms inhabiting this environment negatively. The findings from this study revealed that indigenous bacterial population were able to survive despite crude oil pollution. Also the presence of hydrocarbon utilizing bacteria indicates that these sites habour bacterial strains that can be harnessed and utilized for the degradation of

crude oil derived hydrocarbons. However more study is required to determine the degradative potentials of these bacterial strains so as to utilize them in the remediation of hydrocarbon impacted soils.

References

[1] Barberan, A., Bates, S.T., Casamayor, E.O and Fierer, N. Using network analysis to explore co-occurrence patterns in soil microbial communities. *ISME Journal*, (2012), 6: 343-351

[2] Jiao, S., Liu, Z., Lin, Y., Yang, J., Chen, W., … Wei, G. Bacterial communities in oil contaminated soils: Biogeography and co-occurrence patterns. *Soil Biology and Biochemistry*, (2016), 96: 64-73

[3] Balakrishnan, S., Arunagirinathan, N., Rameshkumar, M. R., Indu, P., Vijaykanth, N., Almaary, K.S and Almutairi, S. M. Molecular characterization of biosurfactant producing bacterium isolated from hydrocarbon-contaminated soil using 16S rRNA gene sequencing. *Journal of King Saud University - Science*, (2022), 34: 101871

[4] Huang, L., Ye, J., Jiang, K., Wang, Y., & Li, Y. Oil contamination drives the transformation of soil microbial communities: Co-occurrence pattern, metabolic enzymes and culturable hydrocarbon-degrading bacteria. *Ecotoxicology and Environmental Safety*, (2021), 225, 112740

[5] Ding, A., Sun, Y., Dou, J., Cheng, L., Jiang, L., Zhang, D., & Zhao, X. Characterizing Microbial Activity and Diversity of Hydrocarbon-Contaminated Sites. *Hydrocarbon*, (2013), 18

[6] Sampson, T., Ogugbue, C.J. and Okpokwasili, G.C. Determination of the Bacterial Community Structure in a Crude Oil – inundated tropical soil using next generation sequencing technique. *Journal of Advances in Microbiology*, (2018), 11 (1): 1-18

[7] Braddock, F. J., Lindstrom, E. J., Reisinger, J. H., and Brown, I. E. Distribution of Hydrocarbon degrading microorganisms in sediments from Prince William Sound, Alaska, following the Exxon Valdez oil spill. *Marine Pollution Bulletin*, (1995), 30: 125-132

[8] Tazaki, C. K., Asada, R., and Kogure, K. Bioremediation of coastal areas 5 years after the Nakhodka oil spill in the Sea of Japan: isolation and characterization of hydrocarbon-degrading bacteria. *Environment International*, (2004), 30: 911-922

[9] Poly, F. S. Attack of the oil-eating microbes. *Well servicing*, (1992), 32(2): 20-28

[10] Rahmeh, R., Akbar, A., Kumar, V., Al-Mansour, H., Kishk, M., Ahmed, N., ... & Al-Okla, N. Insights into bacterial community involved in bioremediation of aged oil-contaminated soil in arid environment. *Evolutionary Bioinformatics*, (2021), 17, 11769343211016887

[11] Chikere, C.B., Okpokwasili, G.C and Chikere, B.O. Bacterial diversity in a tropical crude oil-polluted soil undergoing bioremediation. *African Journal of Biotechnology*, (2009), 8(11): 2535-2540

[12] Stroud, J.L., Paton, G.I and Semple, K.T. Microbe-aliphatic hydrocarbon interactions in soil: implications for biodegradation and bioremediation. *Journal of Applied Microbiology*, (2007), 102: 1239-1253

[13] Bogan, B.W., Lahner, L.M., Sullivan-Beilen, W.R and Paterek, J.R. Degradation of straight chain aliphatic and high-molecular weight polycyclic aromatic hydrocarbons by a strain of *Mycobacterium austroafricanum. Journal of Applied Microbiology*, (2003). 94: 230-239

[14] Bundy, J. G., Paton, G. I., and Campbell, C. D. Combined microbial communitylevel and single species biosensor responses to monitor recovery of oil polluted soil. *Soil Biology & Biochemistry*, (2004), 36: 1149-1159

[15] Majoubi, M., Jaouani, A., Guesmi, A., Ben Amor, S., Jouini, A. and Cherif, A. Hydrocarbonoclastic bacteria isolated from petroleum contaminated sites in Tunisia: isolation, identification and characterization of the biotechnological potential. *New Biotechnology*, (2013), 30(6): 723-733

[16] Maduwuba, M.C., Okpokwasili, G.C and Ibiene, A.A. Bacteriological and heavy metal evaluation of abandoned crude oil-contaminated sites in Gio community, Ogoniland, Nigeria. *GSC Biological and Pharmaceutical Sciences*, (2021), 16(02): 267-273

[17] APHA . Standard methods for the examination of water and waste water (18th ed.). American Public Health Association Inc./ American water works association / Water environment federation, Washington DC. (2005).

[18] Hamamura, N., Olson, S.H., Ward, D.M and Inskeep, W.P. Microbial population dynamics associated with crude oil biodegradation in diverse soil. *Applied Environmental Microbiology*, (2006), 72: 6316-6324

[19] Bates, R.A. Electrometric Determination (1st ed.). John Wiley Sons, Inc., New York. (1954)

[20] Maduwuba, M.C. Mycoremediation of spent lubricating oil contaminated soil using *Pleurotus ostreatus. Nigerian Journal of Microbiology*, (2022). 36(1): 6111-6120

[21] Akpoveta, V.O., Egharevba, F., Medjor, W.O., Osaro, K.I and Enyemike, E.D. Microbial degradation and its kinetics on crude oil-polluted soil. *Research Journal of Chemical Sciences* (2011), 1(6)): 8-14

[22] Leera, S., Ogugbue, C.J and Okpokwasili, G.C. Inherent bacterial diversity and enhanced bioremediation of an aged crude oil contaminated soil in Yorla, Ogoniland using composted plant biomass. *Journal of Advances in Microbiology*, (2018), 9(3): 1-11

[23] Chikere, C.B and Ekwuabu, C.B. Molecular characterization of autochthonous hydrocarbon utilizing bacteria in oil-polluted sites at Bodo community, Ogoniland, Niger Delta, Nigeria. *Nigerian Journal of Biotechnology*, (2014), 27: 28-33

[24] Ramadass, K., Smith, E., Palanisami, T., Mathieson, G., Srivastava, P., Megharaj, M., & Naidu, R. Evaluation of constraints in bioremediation of weathered hydrocarbon-contaminated arid soils through microcosm biopile study. *International journal of environmental science and technology*, (2015), 12, 3597-3612

[25] Eze, V.C and Okpokwasili, G.C. Microbial and other related changes in a Niger Delta sediment receiving industrial effluent. *Cont. Journal of Microbiology*, (2010), 4: 15 – 24

[26] Chaillan, F., Fleche, A., Bury, E., Phantavong, Y. H., Grimont, P., Saliot, A. and Oudot, J. Identification of biodegradation potential of tropical aerobic hydrocarbon-degrading microorganisms. *Research in Microbiology*, (2004), 155(7): 587-595

[27] Chikere, C.B., Okoye, A.U and Okpokwasili, G.C. Microbial community profiling of active oleophilic bacteria involved in bioreactor-based crude oil polluted sediment treatment. *Journal of Applied & Environmental Microbiology*, (2016), 4(1): 1-20

[28] Galitskaya, P., Biktasheva, L., Blagodatsky, S., & Selivanovskaya, S. Response of bacterial and fungal communities to high petroleum pollution in different soils. *Scientific Reports*, (2021), 11(1), 164

[29] Yi, J., Lo, L.S.H., Liu, H., Qian, P and Cheng, J. Study of heavy metals and microbial communities in contaminated sediments along an urban estuary. *Frontiers in Marine Science*, (2021), 8(741912), 1-17

[30] Ogbo, E.M and Okhuoya, J.A. Bioavailability of some heavy metals in crude oil-contaminated soils remediated with *Pleurotus tuer-regium* Fr. Singer. *Asian Journal of Biological Sciences*, (2011), 4(5): 3, 16

[31] Egobueze, F. E., Ayotamuno, J. M., Iwegbue, C. M., Eze, C., & Okparanma, R. N. Effects of organic amendment on some soil physicochemical characteristics and vegetative properties of Zea mays in wetland soils of the Niger Delta impacted with crude oil. *International journal of Recycling of organic waste in Agriculture*, (2019), 8: 423-435

[32] Ergozhin, Y., Dzhusipbekov, U., Teltayev, B.M., Nurgalieva, G., Shakirova, A., Khudabergenova, k., Izmailova, G and Yelshibayev, N. Crude oil-contaminated soil: Its neutralization and use. *International Journal of Sustainability*, (2020), 12: 30-37.

[33] Maduwuba, M.C and Ibiene, A.A. Effect of *Pleurotus ostreatus* on the polycyclic aromatic hydrocarbon and heavy metal concentrations of lubricating oil-amended soil. *Nigerian Journal of Microbiology*, (2022), 36(2): 6404-6416

[34] Ezekoye, C.C., Chikere, C.B. and Okpokwasili, G.C. Field metagenomics of bacterial community involved in bioremediation of crude Oil – polluted soil. *Journal of Bioremediation and Biodegradation*, (2018), 9:5

[35] Makut, M.D., Oyegue, A.O., Owuna, J and Maichiki, A. Optimization of indigenous hydrocarbon degrading-microbial niche for effective bioremediation of petroleum contaminated soil. *BIOMED Natural and Applied Sciences*, (2022), 1(3): 24-35