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Bacteriological, Chemical and Soil Enzyme Activity Profile of Coastal Wetland Soils Exposed to Crude Oil Exploration in Akwa Ibom State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author OUMJ designed the study, performed the experiments, analyzed and interpreted the data and wrote the first draft of the manuscript. Authors SIE, VON and RAO assisted in the literature and writing of the manuscript. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Coastal wetland soils exposed to crude oil exploration activities were assessed using microbiological and chemical procedures in the wet and dry seasons. The bacteria isolated from the impacted wetland soils included species of the genera *Acinetobacter, Alcaligenes, Citrobacter, Azotobacter, Bacillus, Cellulomonas, Flavobacterium, Micrococcus, Proteus, Pseudomonas, Sarcina, Serratia* and *Staphylococcus.* In addition, species of the genera *Nitrobacter, Nitrosomonas* and *Rhizobium* were isolated from the non-impacted soils. Total heterotrophic bacterial counts (THBC) in the wet and dry seasons ranged from 7.1 \pm 0.4 to 8.6 \pm 0.2 Log₁₀CFU g⁻¹ and 3.7 \pm 0.04 to 5.4 \pm 0.1 Log₁₀CFU g⁻¹ for the control and impacted soils respectively. The counts in control was 1.6 to 1.9 times higher than the impacted soil and the difference was significant at $p = 0.05$. The nitrifying bacteria (NB) associated with the impacted soil were the most adversely affected. There was 2.11 to 139 times higher concentration of the different heavy metals in the impacted soil than the control and 1.13 to 1.26 x 10⁴ times higher Total petroleum hydrocarbon in the impacted soils in

both seasons and these differences were significant ($p = 0.05$). Dehydrogenase activity was 2.8 to 3.1 times higher in the control soils compared to the impacted soil whereas Phenol oxidase was 15.14 to 15.75 times higher in the impacted soils in both seasons. This study indicates that Exploration and Production activitiesi in the coastal wetlands if not carried out according to specified guidelines by the environmental control agencies can result in the reduction of beneficial soil bacterial population and diversity, high concentrations of heavy metals, petroleum hydrocarbon and decreased dehydrogenase activity at impacted explored wetland site. It also revealed nonimpacted regions of this explored wetlands not within oil pipe-line routes are fit for agricultural use.

Keywords: Coastal wetland soils; exploration activity; bacterial count; heavy metal; soil enzyme activity.

1. INTRODUCTION

Crude oil exploration and production activities have led to economic boom in oil producing countries thus providing raw materials for many petrochemical industries and also serve as a source of energy. However, crude oil exploration and production activities often release accidental, deliberate or indiscriminate discharges of crude oil wastes and refined petroleum products into the ecosystem which cause ecological problems of great dimensions [1,2].The type and characteristics of human activities as well as factors or parameters affecting the hydrological regime of wetlands play important role in the balance of the wetland ecosystems. In petroleum producing areas, exploration and production activities can result in frequent oil spills and discharge of wastes which contaminate the environment. This could cause drastic changes in the chemical, microbiological and physical properties of such ecosystem [3,4]. Despite the intense crude oil exploration and production activities in the coastal region of Akwa Ibom State, Nigeria, information revealing the bacteriological, heavy metal and soil enzyme activity status within the area is limited. This has necessitated this study.

2. MATERIALS AND METHODS

2.1 Study Area

The study area is a coastal wetland of Unyenge in Mbo Local Government Area, Akwa Ibom State, Nigeria. Unyenge is located between Latitudes 4° 37' 41.53" and 4° 39'5.01" North and Longitudes 8^0 12'6.18" and 8^0 13'48.71" East (Image 1). The climate is tropical hot humid, characterized by distinct wet (April to October) and dry (November to March) seasons. Rainfall is heavy, over 3000 mm along this coastal fringe. Temperatures are uniformly moderate throughout the year and ranged between 26 and 30° C. There is high relative humidity in the area which enhances the luxuriant growth of plant biomass. The vegetation comprises plant of various kinds such as raphia palm, nypa palm, ferns, shrubs and grasses.

2.2 Sample Collection

Composite soil samples (480) were collected at a depth of 0- 45cm randomly from impacted (soils exposed to Exploration and Production (E and P activities) and control (soils 500 m away from E and P activities) portions of Unyenge coastal wetland. The soil samples were collected with the aid of soil augers into labeled sterile polyethene bags. The samples were taken to the University of Uyo Microbiology and Soil Science Laboratories in ice chamber for bacteriological, chemical and enzyme activity analysis respectively. Sampling was carried out for two wet and two dry seasons (i.e. two years). The dry and wet season sampling were carried out in the months of November to March and May to September respectively. Sampling was done thrice for each season and a total of twelve times during the study. All soil samples for bacteriological analysis were analyzed within 24 hours after collection. Prior to chemical analysis, the samples were air-dried and sieved using 2 mm pore sieve. The sieved soil samples were stored in air tight glass containers and analyzed within two weeks of collection.

2.3 Microbiological Analysis of Soil Samples

2.3.1 Isolation, enumeration, characterization and identification of isolates

Ten-fold serial dilutions of the soil samples were made according to standard procedures [5]. The first ten-fold dilution was made using 10 g of the soil samples in 90 mL of distilled water. The dilution was shaken and further serial ten-fold dilutions made up to 10^8 . Aliquots (1.0 mL and 0.1mL) of 10^{-4} to 10^{-6} dilutions of soil samples were cultured on nutrient agar, nitrate agar, Winogradsky media (I and II), cellulose agar plates (Carboxymethyl cellulose- Congo red agar) and phosphate solubilizing bacteria (PSB) medium (Pikovskaya's agar) plates using the spread and pour plate methods. The inoculated triplicate plates were incubated at $28 \pm 2^{\circ}$ C for 18 to 24 hours for total heterotrophic bacteria, 2 to 14 days for nitrifying, cellulolytic and phosphate solubilizing bacteria. The emerging discrete colonies were counted using a colony counter and the bacterial population expressed in colony forming unit per gram (CFU g^{-1}). Enumeration of the isolates was done only in plates with colonies between 25 and 250. The enumeration of specific bacterial groups (CB and PSB) was based on observation of clear zone around the bacterial colony and the population calculated per gram of soil. The isolates were characterized and identified by comparing to known taxa using Bergey's Manual of Determinative Bacteriology [6].

2.3.2 Hydrocarbonoclastic Organisms

The enumeration of hydrocarbon utilizing microorganisms was done by vapour-phase transfer method as described by [7] using Nigerian light crude as carbon and energy source supplied from the lid of the plates. Aliquots (0.1 mL) of appropriate dilutions (10⁻² to 10⁻⁶) of soil samples were inoculated onto mineral salt medium (MSM) using the surface spreading technique. The medium used for the isolation of oil-degrading bacteria was supplemented with 50μ mL⁻¹ Nystatin to inhibit interfering yeast and mold and pH adjusted to 7.6. Sterile filter papers (Whatman 1) soaked with filter sterile crude oil (Nigerian light crude) were aseptically placed inside the lid of each Petri-dish and inverted over the inoculated plates. These filter papers supplied the hydrocarbon by vapour phase transfer to the inocula Control plates were also prepared without crude oil and incubations made at $28 \pm 2^{\circ}$ C for 5 to 7 days. Colony forming Units (CFU g-1) were enumerated and due number of hydrocarbon-utilizing bacteria calculated by subtracting the number of colony forming units in control from those in test cultures.

2.4 Determination of Heavy Metals

Heavy metals were determined by Atomic Adsorption Spectrophotometer after acid digestion as described by [8,9].

Image 1. Akwa Ibom state showing study area

2.4.1 Digestion of soil

One gram (1 g) each of the air-dried, ground and finely sieved samples was weighed separately into 100 mL digestion flask. Five milliliters (5 mL) of 4M $HNO₃$ was added, followed by 10 mL of 6M HCl. The mixture was heated to dryness on a hot plate maintained at 150° C. The solid mass produced was leached with 10 mL of 4M HCl, filtered into 20 mL standard flasks and the residues washed with more acid and made up to mark.

2.4.2 Determination using atomic absorption spectrophotometer

Reagent blank was prepared, the same way as above, but without sample and the volume made up to 20 mL. The extracts and the blank were analyzed for trace elements using Atomic Absorption Spectrophotometer (UNICAM AA 919).

2.5 Determination of Total Hydrocarbon Content of Soils

The Total Hydrocarbon content of the coastal wetland soils was assessed using Toluene extraction method as described by [10]. 5 g of the soil sample was weighed into a beaker and 10 mL of toluene (Analar grade) added to it. The mixture was shaken vigorously for 5 minutes and allowed to stand for 20 minutes. The mixture then formed 2 layers. The supernatant (toluene – residual oil extract) was introduced into cuvette and the hydrocarbon content (oil) extracted determined spectrophotometrically at 420 nm using Spectronic – 20 Spectrophotometer. The absorbance reading was recorded after reading from a standard curve of the absorbance of different known concentrations of hydrocarbon extractant (toluene). Hydrocarbon concentrations were calculated by multiplying with the appropriate dilution factor and the results expressed as milligrams per kilogram (mg kg^{-1}).

2.6 Soil Enzyme Activities

Dehydrogenase and Phenol oxidase soil enzymatic activities were determined as described by [11].

2.6.1 Determination of Dehydrogenase activity in soils

The Triphenyl tetrazolium Chloride (TTC) method based on the estimation of TTC reduction rate to triphenyl formazan (TPF) in the soil after
incubation was emploved to determine incubation was employed to determine dehydrogenase activity of the soil. Five grams of soil was weighed into test tubes and mixed with 5 mL of TTC dissolved in Tris buffer solution. The tubes were sealed with rubber stoppers and incubated for 24 hours at 30°C. The control containing only 5 mL of Tris-HCl buffer (Hydroxymethyl-aminomethane in distilled water and HCl) without TTC was also prepared and incubated. To each tube, 40 mL acetone was added and shaken thoroughly and further incubated at room temperature for 2 hours in the dark, with shaking at intervals. The soil suspension was then filtered and the optical density of the clear supernatant measured against the blank at 546 nm.

2.6.2 Determination of phenol oxidase activity of soils

One gram (1 g) of soil sample was transferred into a 50 mL measuring flask and mixed with 10 mL of 1% pyrogallol solution. The amended soil was incubated at $30\square C$ for 3 hours after which 0.5 mol L^{-1} ether was added into the mixture in the flask and shaken. The gallic acid produced was extracted with ether and measured spectrophotometrically at 430 nm.

2.7 Statistical Analysis

The results were subjected to analysis of variance (ANOVA) and Kruskal Wallis test on log-transformed data using Statistical Package for the Social Science (SPSS version 20.0, IBM Corp, USA). Results are presented as mean ± standard deviation with levels of significance maintained at 95% for each test.

3. RESULTS

3.1 Bacterial Isolates of Unyenge Coastal Wetland Soils

The bacteria isolates associated with the impacted coastal wetland soils were members of the genera *Acinetobacter, Alcaligenes, Azotobacter, Bacillus, Citrobacter, Cellulomonas, Flavobacterium, Micrococcus, Proteus, Pseudomonas, Sarcina, Serratia and Staphylococcus*. The control soils however harbored in addition to the aforementioned isolates *Nitrobacter, Nitrosomonas* and *Rhizobium.*

3.2 Bacterial Counts

The Total heterotrophic bacteria (THB), Nitrifying bacteria (NB), Phosphate Solubilizing bacteria (PSB), Cellulolytic bacteria (CB) and Hydrocarbon utilizing bacteria (HUB) counts in the wet and dry seasons are presented in Fig. 1. The total heterotrophic bacterial counts in wet and dry seasons for the impacted soil at 5.4 ± 0.3 and 3.9 ± 0.1 Log₁₀CFUg⁻¹ was lower than the control soil at 8.6 ± 0.2 and 7.1 ± 0.4 Log₁₀CFU g^{-1} respectively. The nitrifying bacterial counts 0.6 ± 0.2 and 0.2 ± 0.0 Log₁₀CFU g⁻¹ and hydrocarbon utilizing bacterial counts at 1.4 ± 0.2 and 1.0 \pm 0.1 Log₁₀CFU g⁻¹ were least counts for the impacted and control soils respectively in the wet and dry seasons. The difference in the counts between the microbial groups was significant at $p = 0.05$.

3.3 Chemical Profile of Wetland Soils

The heavy metal concentrations and total petroleum hydrocarbon levels of the wetland soils in the wet and dry seasons are presented in Table 1. Among the heavy metals, Iron at 157.9 ± 0.04 mg kg⁻¹ and 153.8 \pm 0.01 mg kg⁻¹ for the impacted soil was higher than 37.3 ± 0.01 mg kg⁻ and 34.8 ± 0.05 mg kg⁻¹ of the control soils in the wet and dry seasons respectively. The concentration of Nickel, Cadmium and Lead levels was ≤ 0.01 mg kg⁻¹ for the control soils in the wet and dry seasons. Total petroleum hydrocarbon was highest (22.6 \pm 0.4) \times 10³ mg kg⁻¹ and 25.2 ± 0.2) \times 10³ mg kg⁻¹) and least (2.0) \pm 0.06 × 10³ mg kg⁻¹ and 2.0 \pm 0.2 × 10³ mg kg⁻¹) for the impacted and control soils in the wet and dry seasons respectively. Generally, there exist significant ($(p = 0.05)$ differences in the concentrations of the various assessed heavy metals (Fe, Mn, Zn, Cu, Ni, Cr, V, Co, Cd and Pb) and Total petroleum hydrocarbon (TPH) at the sampling locations in both seasons. Correlations showed strong negative linear relationship between TPH and dehydrogenase activity for the impacted soils ($r = -0.66$ and $r = -$ 0.67) in the wet and dry seasons respectively.

3.4 Enzyme Activity Profile of Wetland Soils

The enzyme activity profile of the wetland soils in the wet and dry seasons are presented in Figs. 2 and 3. Dehydrogenase showed increased activity at 43.5 \pm 0.4 and 41.9 \pm 0.1 mg g h⁻¹ in the control soils during the wet and dry seasons. In contrast, the impacted soils showed decreased

dehydrogenase activity of 15.4 \pm 0.07 mg g h⁻¹ in the wet and 13.7 \pm 0.1 mg g h⁻¹ during the dry season. Increased Phenol oxidase activity of 12.6 \pm 0.4 mg g h⁻¹ and 10.6 \pm 0.1 mg g h⁻¹ was observed in the wet and dry seasons for the impacted wetland soils, whereas the control soils show reduced Phenol oxidase activity in the wet 0.8 ± 0.2 mg g h⁻¹ and dry 0.7 ± 0.2 mg g h⁻¹ seasons. Dehydrogenase activity was 2.8 to 3.1 times higher in the control than impacted soils whereas phenol oxidase activity was 15.14 to 15.75 times higher in the impacted soils than the control and these differences were significant at $p = 0.05$. The results indicate a strong positive correlation between dehydrogenase and bacterial densities for the control ($r = 0.84$ and $r =$ 0.81) and impacted ($r = 0.78$ and $r = 0.75$) soils in the wet and dry seasons respectively. However, there was weak to strong negative correlations between phenol oxidase activity and bacterial counts. Strong negative linear relationships ($r = -0.67$ and $r = -0.71$) between phenol oxidase (OX) and THB, OX and NB $(r = -$ 0.47 and $r = -0.48$), OX and PSB ($r = -0.57$ and $-$ 0.69), OX and CB (r = $-$ 0.69 and r = $-$ 0.74) and weak negative linear relationship ($r = -0.23$) and $r = -0.24$) between OX and hydrocarbon utilizing bacteria in the wet and dry seasons respectively.

4. DISCUSSION

The bacteriological assessment of Unyenge coastal wetland soils has revealed the association of various genera of bacteria. These included species of the genera *Acinetobacter, Alcaligenes, Citrobacter, Azotobacter, Bacillus, Cellulomonas, Flavobacterium, Micrococcus, Proteus, Pseudomonas, Sarcina, Serratia*, *Staphylococcus, Nitrobacter, Nitrosomonas* and *Rhizobium.* These bacteria are such that play critical roles in biodegradation, biogeochemical cycling, bioremediation and bio-control activities in the environment [4,12]. The existence of the bacteria however varied with the sampling location; species of the genera *Nitrobacter, Nitrosomonas* and *Rhizobium* were not isolated from the impacted soils. This variation could be attributed to the impacting degree of the crude oil exploration and production activities on the coastal wetland soils [1,3,4,13]. This study also revealed higher bacterial counts for the control than impacted soils in the wet and dry seasons (Fig. 1). This trend in microbial counts is attributed to the effects of the crude oil Exploration and Production (E & P) activities such as spillage and leakage of crude oil, used *John et al.; CJAST, 40(16): 1-11, 2021; Article no.CJAST.69984*

petroleum products, drilling fluid and drilling wastes on the physicochemical characteristics of the soil which in turn affects the soil microbial population. Crude oil E & P processes contributes to localized loading of Total petroleum hydrocarbon (TPH) into the environment through accidental spillage or leaks of oil from production wells, storage tanks, gathering lines, transportation lines and pits. Petroleum hydrocarbons entering the wetlands is adsorbed onto the organics and accumulation in the soils disrupt the ecosystem dynamics by altering the mineralization rate of soil organic matter and nutrient cycling. Also, the increase in Total Petroleum Hydrocarbon in the soil induced lowering of soil pH (i.e. increase acidity) because of the production of acidic intermediates by hydrocarbonoclastic organisms during biodegradation process [12,14]. Wastes generated during the E and P activities consist of hydrocarbon compounds, acids and heavy metals. These constituents influence the soil physicochemical characteristics, increase acidity, toxicity and reduce nutrient and oxygen availability which in turn affect the microbial population [15,16].

The dispersion of hydrocarbons and other pollutants by surface water run-off from oil well zones and oily waste dumpsites contributed to the reduction in microbial population. The soils of the impacted and non-impacted coastal wetlands of Unyenge exhibited higher bacterial counts in the wet season than dry season for all groups of bacteria. The low variations in the bacterial counts of the various groups is attributed to changes in the properties of the wetland soils. The higher bioload in the wet season is attributed to mitigation by surface water runoff that increased organic matter, dilute and neutralize the soil acidity. Similar seasonal difference in microbial counts has been reported for soil microorganisms [3,17] and wetland soil microbial population [18].

Total Petroleum Hydrocarbon (TPH) level of the impacted wetland soils revealed slight decrease in the dry season compared to the wet season (Table 1). The decrease could be attributed to higher biodegradative activities in the dry season due to photo-oxidation of the petroleum hydrocarbon pollutants at the explored sites [12]. The global average permissible limit of TPH for soil is 1,000 mg kg^{-1} [19]. The results of this study indicate that impacted soils of the coastal wetland exhibited TPH level higher than the global permissible limit in soils in the wet and dry seasons. The effects of TPH levels above permissible limits in the soil include disruption of the soils dynamics by altering the mineralization rate of soil organic matter and resultant nutrient remineralization rates. This results in the alteration of the soils microbiological, physical as well as chemical characteristics. High concentrations of hydrocarbons in soils also reduce growth, stem height, density, live percent cover and above ground biomass of plants [1,16,19,20,21].

Key: IMP - Impacted soils, CON - Control soils, W - Wet season, D- Dry season, THB - Total heterotrophic *bacteria, NB – Nitrifying bacteria, PSB – Phosphate solubilizing bacteria, CB – Cellulolytic bacteria, HUB – Hydrocarbon utilizing bacteria*

Table 1. Chemical profile of wetland soils

KEY: IMP – Impacted soils, CON – Control soils, W – Wet season, D- Dry season, BDL-Below Detectable Limit (<0.01 mg kg⁻¹)

Fig. 2. Dehydrogenase activity of wetland soils (wet and dry seasons) *Key: IMP – Impacted soils, CON – Control soils, W – Wet season, D- Dry season*

Fig. 3. Phenol oxidase activity of wetland soils (wet and dry seasons) *Key: IMP – Impacted soils, CON – Control soils, W – Wet season, D- Dry season*

Heavy metals are considered serious pollutants because of toxicity, persistence and nonbiodegradable conditions in the environment and constitute a threat to humans and other biological life [12,22]. The results indicate that heavy metal concentrations were higher in impacted soils compared to the control soils in the wet and dry seasons. The trend of heavy metal concentrations was in the order: Fe > Zn > V > N > Cu > Cr >Mn > Co> Pb > Cd for impacted soils (Table 1). The varying concentrations of heavy metals for the impacted and non-impacted soils suggests the influence of soil properties, metal properties and environmental factors such as run-off that washed the E and P wastes into the wetlands. Heavy metal contamination of the soil

from petroleum hydrocarbons have also been reported in several studies [1,14].

Iron (Fe) constitute one of the eight micronutrients required for plant and microbial growth. It is involved in chlorophyll synthesis, oxidation-reduction in respiration and also forms the constituents of certain enzymes and proteins in plants and microbes [23]. The iron concentration in both wet and dry seasons were high. (Table 1) and corroborates the findings in other studies that reported high levels of iron in Nigerian soils with a critical level of 4.5 mg kg^{-1} [24]. The concentrations of iron in the soils under study were higher than the critical value in the wet and dry seasons. This result followed same trends as reported elsewhere [18,24] for Akwa Ibom State wetland soils. Manganese (Mn) is also essential for the growth of plants and soil microbes. It functions in nitrogen and inorganic acid metabolism, carbon(iv)oxide assimilation, carbohydrate breakdown, formation of carotene, riboflavin and ascorbic acid [23]. The critical value for Manganese is 1.0 mg kg^{-1} . The concentrations of Manganese for the soils were all in excess of the critical value. Studies indicate that Manganese concentrations for Akwa Ibom State wetland soils are higher than critical value [24]. The results of this study agree with the reports of Manganese concentrations for Akwa Ibom State wetland soils.

Enzyme activities play the key roles in the biochemical functioning of soils, including soils organic matter formation and degradation, nutrient cycling and decomposition of xenobiotics [12]. Dehydrogenases are intercellular enzymes that catalyze oxidation-reduction reactions required for the respiration of organic compounds and is considered a measure of microbial activity. There was increase in dehydrogenase (DH) values of the control compared to the impacted soils in both seasons which positively correlated with bacterial counts of the different groups (i.e. THB, NB, PSB and CB) in the soils. The low DH values of the impacted soils is attributed to the influence of increased Total petroleum hydrocarbon and heavy metal levels in the soils. Reports indicate that increased petroleum hydrocarbon and heavy metal concentrations in soils exhibit inhibitory activity on soil dehydrogenase [25].The results of dehydrogenase activities for this study corroborates with other studies [17,25].Microbial Phenol Oxidases mediate biogeochemical processes in soils, including microbial acquisition of Carbon, Nitrogen, Lignin degradation, Carbon

mineralization and sequestration and dissolve organic carbon export. Phenol Oxidases are involved in the breakdown of lignin, other aromatic compounds and in the production and degradation of humic substances. They oxidize phenolic compounds using Oxygen as an electron acceptor .The Phenol oxidase activity (OX) for impacted soils was higher than the control in both seasons. This results also corroborates with the studies of [17].

5. CONCLUSION

Wetlands in Akwa Ibom State are traditional sources of fishes, firewood, medicines, timber and wildlife. They are more important than the upland farmlands because they are used in the wet and dry seasons and all year round for crop production. The wetland therefore forms an important link in the economic system of the state. However, crude oil exploration and production activities can cause changes in the bacteriological, chemical and enzyme activity characteristics of impacted coastal wetland soils and render them unsuitable for agricultural purposes.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

- 1. Wang X, Feng J, Zhao J. Effects of crude oil residuals on soil chemical properties in oil sites, momoge wetland, China. Environmental Monitoring Assessment. 2010;161: 271–280.
- 2. Ita AE, Ibok UJ, Ita MU, Peters SW. Petroleum exploration and production: Past and Present Environmental Issues in Nigeria's Niger Delta. American Journal of

Environmental Protection*.* 2013;1(4):78– 90.

- 3. Nwaugo VO, Onyeagba RA, Chima GN, Agwaraze DI. Effects of Drilling Wastes on the Farmland Microbial Spectrum in Egbema, South-South Nigeria Community. Research Journal of Microbiology*.* 2007; 2(8):632–638.
- 4. Bahrampour T, Moghanlo VS. Evaluation of soil biological activity after soil contamination by crude oil. International Journal of Agriculture:Research and Review. 2012;2(6):671–679.
- 5. Etok CA, Udo SM, Eja ME. General microbiology practical manual. Abison. Printing Press, Calabar, Nigeria; 2004.
- 6. Holt JG, Kreig NR, Sneath PHA, Staley JT, Williams ST. Bergey's Manual of Determinative Bacteriology (9th Edition) Williams and Wilkins Publishers, Baltimore, USA; 1994.
- 7. Eziuzor SC, Okpokwasili, GC. Bioremediation of Hydrocarbon Contaminated Mangrove Soil in a Bioreactor. Nigerian Journal of Microbiology.2009;23(1):1777–1971.
- 8. Udo, EJ, Ibia, TO, Ogunwale, JA, Ano, AO, Esu, IE. Manual of Soil, Plant and Water Analysis. Sibon Books Limited, Lagos; 2009.
- 9. Association of Official Analytical Chemists (AOAC). Official Methods Of Analysis (19th Edition) AOAC. Washington DC.; 2012.
- 10. Wemedo SA, Obire O. Acute toxicity test of oil field waste water on bacterial community of a soil in Nigeria. Research Journal of Environmental Toxicology. 2012;6(1):25–32.
- 11. Alef K. Dehydrogenase activity. In: Alef K,Nannipieri P. editors. Methods in soil microbiology and biochemistry. Academic Press Ltd. 1995;228-231.
- 12. Mohapatra PK. Textbook of environmental microbiology. I. K. International Publishing House Pvt. Limited, New Delhi. 2008;128– 484.
- 13. John, RC, Akpan, MM, Essien, JP, Ikpe, DI. Fate of Nitrogen – Fixing Bacteria in Crude Oil Contaminated Wet Ultisoils. Bulletin of Environmental Contamination and Toxicology. 2011; 87(37):343–353.
- 14. Basumatory B, Bordoloi S, Sarma HP, Das CH. A study on the physicochemical properties and heavy metal content in crude oil contaminated soil of duliajan, Assam, India. International Journal of

Advanced Biological Research*.* 2012; 2(1):64–66.

- 15. Onwukwe SI, Nwakaudu MS. Drilling Wastes Generation and Management
Approach. International Journal of Approach. International Journal of Environmental Science and Development*.* 2012;3(3):252–257.
- 16. Pathak H, Bhatnagar K, Jaroli DP. Physicochemical Properties of Petroleum Polluted Soil Collected from Transport Nagar (Jaipur). Indian Journal of Fundamental and Applied Life Sciences. 2011;1(3):84–89.
- 17. Nwaugo VO, Onyeagba RA, Azu N, Nworie O. Petroleum produced (formation) water induced changes in bacterial quality and soil enzymatic activities in a Farmland in Egbema, Southern, Nigeria. Estud. Biol. 2007;66:89–97.
- 18. John OUM, Eduok SI. Microbiological, physicochemical and enzyme activity profile of ayadehe coastal wetland soils, Nigeria. Journal of Scientific Research & Reports. 2018;20(2):1-11.
- 19. Lotfinasabasi S, Gunale VR, Rajurkar NS. Petroleum hydrocarbon pollution in soil and its bioaccumulation in mnagrove species, avicernia marina from alibaug mangrove ecosystem, Maharashtra, India. International Journal of Advancements in Research and Technology. 2013;2(2):1–7.
- 20. Essien OE, John IA. Impact of crude oil spillage pollution and chemical remediation on agricultural soil properties and crop growth. Journal of Applied Science and Environmental Management. 2010;14(4):147–154.
- 21. Shukry WM, Al-Hawas GHS, Al-Moakal RMS, El-Bendary MA. Effect of petroleum crude oil on mineral nutrient elements, soil properties and bacterial biomass of the rhizosphere of jojoba. British Journal of Environment and Climate Change. 2013;3(1):103–118.
- 22. Nwauche CO, Ugoji EO. Effects of Heavy Metal Pollution on the Soil Microbial Activity. International Journal of Environment, Science and Technology. 2008;5(3):409–414.
- 23. Brady NC, Weil RR. The nature and properties of soils (14th Edition) Prentice Hall, New Jersey. 2008;1–980.
- 24. Udoh BT, Ibia TO, Udo BU, Edem SO. Assessment of micro-nutrient status of inland depression and flood plain (Wetland). Soils in Akwa Ibom State South – Eastern Nigeria. Journal of Tropical

Agriculture, Food, Environment and Extension*.* 2008;7(2):156 –161.

25. Akubugwo EI, Ogbuji GC, Chinyere CG, Ugbogu EA. Physicochemical properties

and enzymes activity studies in refined oil contaminated soil in Isiukwato, Abia State, Nigeria. Biokemistri*.* 2009;21(2):79– 84.

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