PRINT ISSN 1119-8362
Electronic ISSN 2659-1502

Full-text Available Online at
https://www.ajol.info/index.php/jasem
http://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 27 (2) 361-366 February 2023

Influence of Water Insoluble Fractions of Crude Oil on Physicochemical Properties and Microbes of Soil Obtained From a Tertiary Institution in Benin City, Nigeria

***[1]OGBEIDE, GE; [2]ERIYAMREMU, GE**

**[1]Department of Environmental Management & Toxicology, [2]Department of Biochemistry, University of Benin, Benin City, Nigeria*

**Corresponding Author Email: grace.uhunamure@uniben.edu; Tel: +2348132521152*

ABSTRACT: The global use of petroleum hydrocarbon for domestic and industrial purposes has necessitated the occurrence of the contamination of the living and non-living components of the environment. This study investigated the influence of water insoluble fractions of crude oil on physicochemical properties and microbes of soil obtained from a tertiary institution in Benin City, Nigeria using standard procedures. The result for soil physicochemical properties showed a significant increase ($p \leq 0.05$) in total hydrocarbon, nitrate, phosphate and sodium ion, however nitrate ion decreased in day 28 and 42. Soil pH, electrical conductivity, calcium, potassium and magnesium ion concentration significantly decreased, although with very minute variations. Soil bacteria and fungi count also decreased significantly ($p \leq 0.05$), with some variation in fungi count ($p \leq 0.05$). The result suggest that treatment with water insoluble fraction of crude oil induced changes in the soil physicochemical and microbial component, which infers alterations in soil structure and component. This could negatively affect plant cultivation and growth, as well as animals and human that consume contaminated plants. Oil contamination should therefore be avoided or properly managed to prevent its detrimental effect on the non-ling and living soil component.

DOI: https://dx.doi.org/10.4314/jasem.v27i2.26

Cite this paper as: OGBEIDE, G. E; ERIYAMREMU, G. E. (2023). Influence of water insoluble fractions of crude oil on physicochemical properties and microbes of soil obtained from a tertiary institution in Benin City, Nigeria. *J. Appl. Sci. Environ. Manage.* 27 (2) 361-366

Dates: Received: 26 January 2023; Revised: 11 February 2023; Accepted: 14 February 2023
Published: 28st February 2023

Keywords: Water insoluble fraction, Oil spillage, Soil Physiochemical properties, Bacteria, Fungi

Worldwide, crude oil products are extensively used in the generation of fuel for both domestic and industrial use; this is due to the increasing daily demand of energy (Mohan, 2018). These products such as gasoline, petroleum, kerosene, diesel and natural gas are used as fuel for different purposes, ranging from kerosene and natural gas for cooking, petroleum and diesel to power vehicles and automobiles and gasoline for recreational vehicles and boats etc (Ahmed and Fakhruddin, 2018). Crude oil products can be separated into their water-soluble and insoluble components; water-soluble fraction is the fraction that dissolves readily in water, while water insoluble fraction does not dissolve readily in water (Edema, 2008). These components are distinct in their chemical components and so is their impact on the environment (Ahmed and Fakhruddin, 2018). A key consequence of oil exploration is spillage, oil spillages could be of negative environmental impact, and this impact affects the components of the environment such as soil, plants, animals and Man (Kuch and Bavumiragira, 2019). Soil is a very important layer of the lithosphere that is biologically active with very environmentally significant functions that include a. Maintaining habitat for microorganisms and other animals (Nieson et al., 2015) (b), Biomass production, which is the basis for animals and human nutrition (Diaz et al., 2018; Silver et al., 2021) (c). Participation in the process of mineralization, filtration and buffering, d. Retention of ions, gases, microorganisms and particle suspension (Brevik and Sauer, 2015). The soil also acts as a neutral filter; it stores and transforms harmful

**Corresponding Author Email: grace.uhunamure@uniben.edu; Tel: +2348132521152*

substances produced from human activities. The release of petrol into the environment may occur during loading, transportation and unloading processes, other causes include war, sabotage, natural disaster and tanker failure (Egbe and Thompson, 2010; Jernelöv, 2010). It causes changes in the physical and chemical properties of the soil and could have a phytotoxic effect on the germination of seed, growth and yield. It could also affect water, oxygen and nutrient content of the soil (Abii, 2009; Henry, 2005). Microorganism are involved in soil contamination process; petroleum toxicity could result in the destruction of these organism especially at high concentration, however, hydrocarbon degrading bacteria and fungi are involved in breaking down these hydrocarbon (Ogbeide et al., 2019). This process is termed bioremediation, bioremediation transforms the toxic substances to harmless products such as CO_2, H_2O and fatty acids (Ahmed and Fakhruddin, 2018). Bacteria with genera, Bacillus, Pseudomonas Nocardia, Escherichia, Staphylococcus, Flavobacterium, Enterobacter and Acinetobacter can degrade hydrocarbons (Borowik, 2020). Some fungi specie such as Aspergillus, Penicillium, Fusarium, Candida and Cladosparium have also been isolated from marine sediments of the Niger Delta and they are known to degrade hydrocarbons (Mansi et al., 2018). The role of indigenous microbes in hydrocarbon degradation cannot be over-emphasized, as immediately contaminants are present in a site there is a remarkable change in the microbial composition of that site; The microbes increase in their number and decreases itself naturally once the contaminants are degraded (Mansi et al., 2018).

Crude oil based hydrocarbons are made up of aromatic and aliphatic hydrocarbons with varying hydrocarbon length, the degradation of alkanes is more rapid followed by simple aromatic hydrocarbons such as toluene, benzene, xylene-isoalkane while other aromatics and cycloalkanes degrade more slowly (Nzili, 2018). There is a wide variation in the rate degradation of the different classes of organic compounds (Nzili, 2018). Non-hydrocarbon compounds, including nitrogen and Sulphur, these contaminants are known to change the water holding capacity and hydrophobicity (Eldos et al., 2022). Due to the report from a number of studies on the toxic effect of crude oil on the soil components of the environment and the scare studies on its water insoluble fraction, the need for this study became necessary. This study therefore investigated the influence of water insoluble fractions of crude oil on physicochemical properties and microbes of soil obtained from a tertiary institution in Benin City, Nigeria.

MATERIALS AND METHODS

Sample collection: The research was carried out in the department of biochemistry of the University of Benin, Benin City, Nigeria. Sandy loamy soil sample was collected from a serene region of the University with very minute vehicular disturbance, soil was sieved and 500g was measured into nursery bags after air-drying was while crude oil was collected from the Warri refinery.

Fractionation of crude oil: A portion of the crude oil collected was fractionated in accordance with Anderson et al. (1974) procedure. Crude oil (300 ml) and distilled water (600 ml) was measured in a 1 litre conical flask and stirred consistently for 48 h with a magnetic stirrer. Water insoluble fraction was separated from water soluble fraction and collected in a conical flask using a separating funnel that was set up for 48 h.

Soil treatment: The soil was treated with varying concentration of WIF, 0, 2, 5, 10 and 20% at time interval, 0, 14, 28 and 42 days, which was selected after a range-finding test was done.

Soil physicochemical properties: Soil physicochemical analysis was performed using 2 gram WIF treated and control samples, samples were then air-dried, sieved through a 2mm sieve and stored in a sterile plastic container, ready to be analyzed. All parameters such as soil hydrocarbon contents (THC), Nitrate, Phosphate, calcium ion (Ca^{2+}), Potassium ions (K+), Sodium ion (Na+), Electricity Conductivity (EC) and Magnesium ion (Mg2+) were determined colorimetrically according to AOAC, (1984). pH on the other hand was done according the procedure of Bates (1954).

Bacteria identification and enumeration: Bacteria isolates identification was performed based on microscopic, cultural and biochemical characteristics according to Bergey's manual of determinative bacteriology (Brown, 1939). Analysis was performed after sterilization of all equipment and glassware. Glassware were sterilized in a hot air at oven at 160^0C for 2 h. while growth media and distilled water were autoclaved at 120^0C for 15mins. Soil sample (1 gram) suspended in 10mls sterile water was used to prepare concentrated solution and the suspension was serially diluted in the order of 10^2, 10^4, 10^6, 10^8, 10^{10} concentrations. Heterogeneous bacteria count was estimated in triplicates using pour plate method with concentrations 10^6, 10^8 and 10^{10}. Bacteria was isolated using nutrient agar with the presence of nystatin (0.015% w/v) to inhibit the growth of fungi and incubation was done for 7 days at ambient temperature. The pure isolates were maintained slantly on agar at 4^0C.

Enumeration, isolation and identification of fungi: Water insoluble fraction contaminated and control soil samples each, was diluted in sterile water in a concentration ratio of one (1 g) to 9 ml while a tenfold serial dilution of concentrate was made in a ratio of 1:10 to 10,000. An aliquot of 0.1 ml from the 10^{-4} dilution was plated in triplicates for each sample, on PDA, potato dextrose agar that was amended with the antibiotics streptomycin, which helps to inhibit bacteria growth, using pour plate method. The plates were incubated for 72 h at 27°C, the number of viable fungi in the WIF and control soil samples were calculated, the number of colonies formed. The volume of inoculum and dilution factors were expressed in colony forming unit per gram. Preparation and identification of fungi specimens was carried out under a light microscope through morphological and taxonomical characteristics (Barnett and Hunter, 1998).

Statistical analysis: Data was collected and analysis was performed using graph pad prism 6 and the results were represented as mean ±standard error (SEM). One way ANOVA was carried out for comparing three and more parameters while turkey test was done for significant difference set at $p \leq 0.05$.

RESULTS AND DISCUSSION

Human activities has greatly contributed to the generation and distribution of petroleum products as a source of fuel while simultaneously causing environmental menace due to spillage. This research was undertaken to investigate the effect of crude oil water insoluble fraction on soil physicochemical and microbial components. The data from this study revealed that WIF treatment resulted in a significant increase ($p \leq 0.05$) in the total hydrocarbon, nitrate, phosphate and sodium concentration, however nitrate concentration decreased at day 28 and 42 for all concentrations, except at 20% where no significant changes was observed (Fig. 1A, B, C and D).

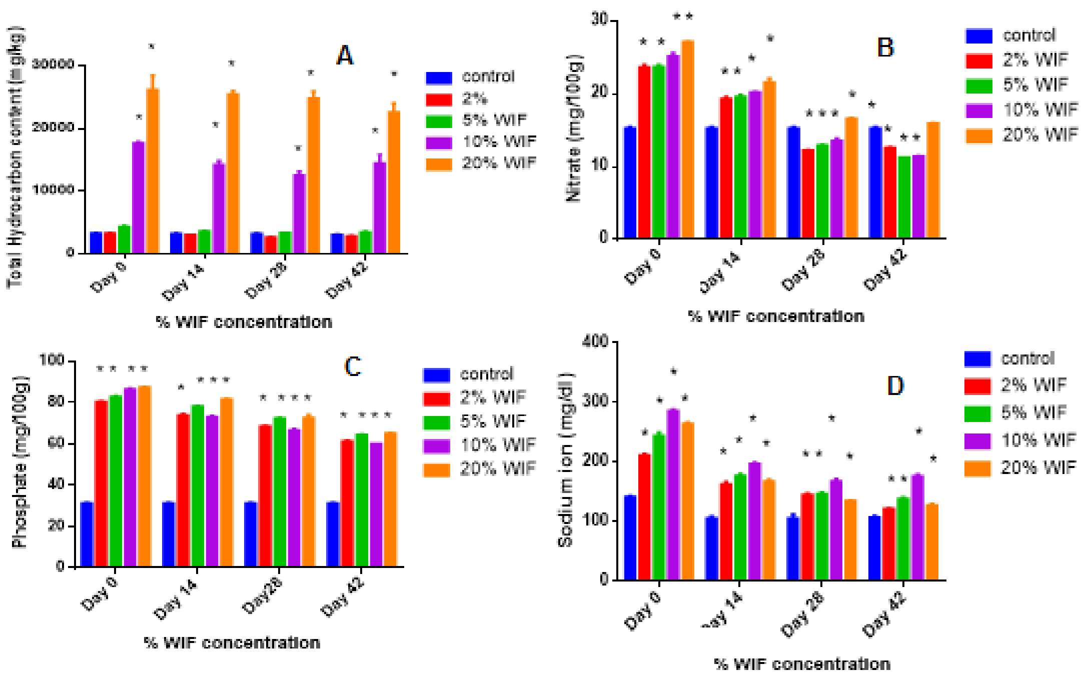

Fig. 1. A. Total hydrocarbon content. B. pH levels. C. Nitrate. D. Phosphate ion. Data presented as mean ± SEM of three determinations and * indicates significant difference at $p \leq 0.05$.

Meanwhile, a significant decrease ($p \leq 0.05$) was noticed with soil pH, electrical conductivity, calcium, potassium and magnesium ion concentration when compared with control, although with some similar variations in pattern, an increase that was significant ($p \leq 0.05$) was first observed in most concentrations of day 0 (Fig. 2A, B, C, D and E). Obire et al., (2008) report that whole crude contamination significantly increased organic carbon, sodium ion, ammonium and nitrate ion. Meanwhile other soil parameters such as pH, electrical conductivity (E.C), potassium, magnesium ion and available phosphorus reduced. This is in line with the present studies although with very minute variations. Curylo et al., (2020), have reported similar results; significant changes were seen in soil organic compound, nitrogen content and phosphatases. A good number of research have also reported similar findings on the effect of crude oil on soil THC, pH and nitrate (Anacletus *et al.*, 2017, Pathak *et al.*, 2011, Hassan *et al.*, 2018). This again confirms the result of this research, although the previous research studied the effect of whole crude, which was seen to have very similar effect with WIF that was investigated in this research.

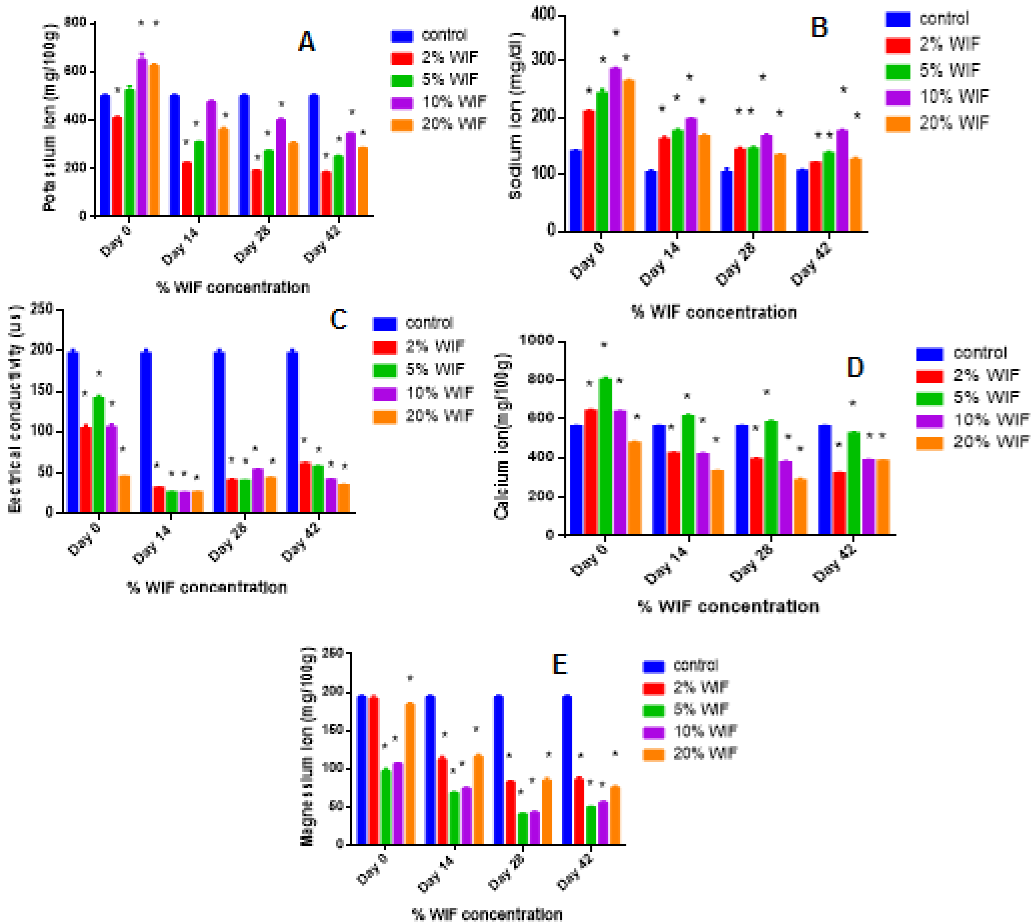

Fig. 2. A. Electrical conductivity. B. Sodium ion C. Potassium ion D. Calcium. E. Magnesium ion. Data represented as mean ± SEM of three determinations and * indicates significant difference at $p \leq 0.05$

The present study also reported that WIF reduced soil bacteria and fungi count. Bacteria analysis that entailed the identification and quantification showed that *Pseudomonas spp*, *Bacillus subtilis*, *Staphylococcus aureus*, *Enterobacter spp* and *klebsiella spp* were present in the soil. It also revealed that the total count of heterogeneous bacteria ranged from 0.06×10^4 - 9×10^8 in control and treated plants samples. However just *Pseudomonas spp* and *Bacillus subtillis* were able to strive on day 42, although insignificantly, a similar result was observed in previous study (Omotayo *et al.*, 2012, Ekanem and Ogunjobi, 2017). In Ekanem and Ogunjobi, (2017), *Pseudomonas spp* and *Bacillus subtillis* showed the highest hydrocarbon utilization when compared with *Enterobacter* spp. This implies that only the crude oil degraders of very high potentials (*Bacillus spp. and Pseudomonas spp*.) were able to survive at very toxic levels of contamination. Similar results have been observed in previous studies (Sadoun *et al.*, 2008; Ekanem *and* Ogunjobi *et al.*, 2017 and Inieke *et al.*, 2018). Fungi analysis in this present studies showed presence of some hydrocarbon degrading genera; *Penicillium*, *Aspergilius*, *Trichoderma* and *Mucor*, which could account for the slight increase in fungi count, since these genera can strive in oil contaminated areas . It was also revealed that as concentration and time of crude oil contamination increased, fungi species decrease significantly especially on day 42. This has been reported in previous studies with water-soluble fraction and crude oil as toxicant (Ai-Jawhari, 2014, Dawoodi *et al.*, 2015, Al-Nasrawi, 2012), however WIF has not been previously reported. The finding from the present indicated that WIF contamination resulted in alteration in soil physicochemical and microbial component.

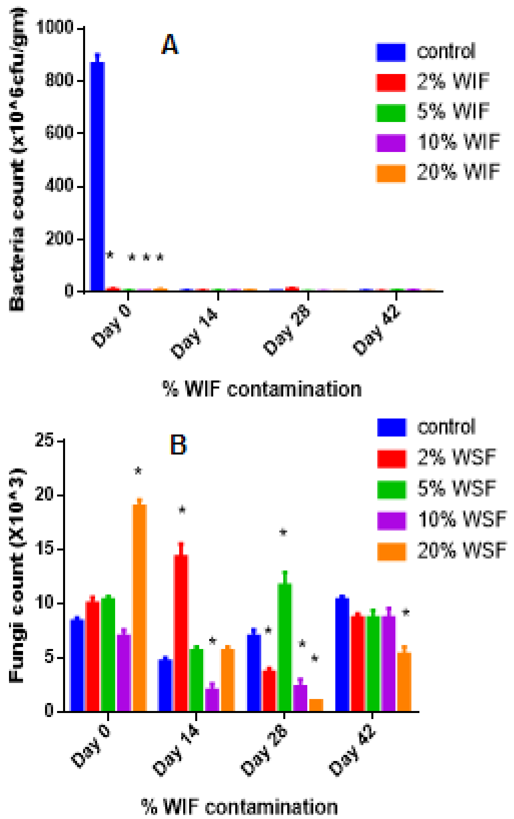

Fig 3. A. Bacteria count B. Fungi count. Results are presented as mean ± SEM of three determinations and * indicates significant difference at $p \leq 0.05$

Fig 3. A. Bacteria count B. Fungi count. Results are presented as mean ± SEM of three determinations and * indicates significant difference at $p \leq 0.05$

Conclusion: The findings from the present research demonstrated that soil treatment with the water insoluble fraction of crude oil resulted in alterations in the soil physicochemical, bacteria and fungi components. This could have a direct effect on the soil integrity, causing alterations in the normal enzymatic interaction and nutrient availability for maximum crop yield. WIF contamination should however be checked to prevent the risk it poses to the living and non-living structure of the environment.

Acknowledgement: TETFUND; my sincere appreciation goes to TETFUND fund (2022 intervention) for funding this research.

REFERENCES

Abii, PC (2009). The Effect of Oil-Spillage on the Soil of Eleme in Rivers State of the Niger Delta Area of Nigeria, *Res J Environ Sci*. 3: 316-320.

Ahmed, F; Fakhruddin ANM (2018). A Review on Environmental Contamination of Petroleum Hydrocarbons and its Biodegradation. *International Journal of Environ Science Nat Res*. 11(3):63-69.

Al-Jawhari, IFH (2015). Ability of some fungi isolated from a sediment of Suq-Al Shuyukh marshes on biodegradation of crude oil. *International Journal of Current. Microbiology and Applied Science*. 4(1): 19-32.

Al-Nasrawi, H (2012). Biodegradation of crude oil by fungi isolated from gulf of Mexico. *Journal of Bioremediation and Biodegradation*. 3(4): 1-6.

Anacletus, FC; Nwauche, KT; Ighorodje-Monago, CC (2017). Effect of triton X-100 and white rot fungus (*Pleurotus ostratus*) on physico-chemical composition of crude oil impacted soil. *Journal of Applied Life Sciences International*. 12(3):1-7.

Anderson, JW; Neff, JM; Cox, BA; Tatem, HE; Hightower, GM (1974). Characteristics of dispersions and water-soluble extracts of crude and refined oils and their toxicity to estuarine crustaceans and fish. *Marine Biology*. 27(1): 75-88.

AOAC (1984). *Standard official methods of analysis of the association of analytical chemists.* 14 Edn. Association of Analytical Chemists, Washington DC. pp 121.

Bates, RA (1954). Electrometric determination. John Wiley and Sons, Inc. New York.

Barnett, HL; Hunter, BB (1998). Illustrated genera of imperfect fungi. American Phytopathological Society. America, APS Press. pp 218

Borowik, A; Wyszkowska, J; Kucharski, M; Kucharski, J (2020). The role of Dactylis glomerata and diesel oil in the formation of microbiome and soil enzyme activity. *Sensors*. 20:33-62.

Brevik, EC; Sauer, TJ (2015) The past, present, and future of soils and human health studies. *Soil*. 1:35–46.

Brown, JH (1939). Bergey's Manual of Determinative Bacteriology (5th ed.). *Americ. J. of Public Health and the Nation's Health*. 29(4): 404–405.

Curyło K; Telesinski A; Jarnuszewski, G; KrzyskoŁupicka, T; Cybulska K (2020). Analysis of Chemical and Biochemical Parameters of Petrol-Contaminated Soil after Biostimulation with an Enzyme Reagent. *Processes*. 8: 949

Dawoodi, V; Madani, M; Tahmourespour, A; *and* Golshani, Z (2015). The study of heterotrophic and crude oil-utilizing soil fungi in crude oil contaminated regions. *J. Bioremed. Biodegrad*. 6(2): 270-275.

Díaz, S; Pascual, U; Stenseke, M; Martín-López, B; Watson, RT; Molnár, Z; Hill, R; Chan, KMA; Baste, IA; Brauman, KA (2018). Assessing nature's contributions to people: Recognizing culture, and diverse sources of knowledge, can improve assessments. *Sci.* 359: 270–272.

Edema, NE; Okoloko, EG; Agbogidi, OM (2008). Physical and ionic characteristics in water soluble fraction (WSF) of Olomoro well-head crude oil before and after exposure to *Azolla africana* Desv. *Afr. J. Biotechnol*. 7(1): 35-40.

Ekanem, JO; Ogunjobi, AA (2017). Hydrocarbon degradation potentials of bacteria isolated from spent lubricating oil contaminated soil. *J. Appl. Sci. Environ. Manage.* 21: 973-979.

Eldos, HI; Khan, M; Zouari, N; Saeed, S; Al-Ghouti, MA (2022). Characterization and assessment of process water from oil and gas production: A case study of process wastewater in Qatar. *Chem. Environ. Engineer*. 6:1-9.

Egbe RE; Thompson D (2010). Environmental challenges of oil spillage for families in oil producing communities of the Niger Delta region. *JHER [Internet*]. 13:24-34.

Hassan, KA; Sani, Y; Ajoke, OY; Ajisafe, OJ (2018). Bacteriological and physicochemical properties of petroleum-contaminated soil collected from a mechanic site in Abuja, North-Central Nigeria. *Biotechnology*. 3(1): 1-6.

Henry, JGH (2005). Environmental Science and Engineering. G W. pp 64-84.

Jernelöv, A (2010). The threats from oil spills: now, then, and in the future. *Ambio*. 39(5):353-366.

Inieke, ES; Michael, EI; Ben, MG (2018). Microbial remediation of crude oil contaminated soil using animal waste (chicken droppings and cow dung) with degrading potentials. *Toxicol Open Access*. 4 (1): 135-138.

Kush, SG; Bavumiragira, JP (2019). Impacts of crude oil exploration and production on environment and its implications on human health: South Sudan Review. *International J Sci. Res. Pub.* 9(4): 247-256.

Mansi, E. W; Akaranta, O; Abu, G. (2018). Screening of hydrocarbon degrading fungi in crude oil polluted soil isolated in the Niger Delta. *Afr. J. Environ. Sci. Technol*. 12(5):172-176.

Mohan, M. (2018). Perovskite Photovoltaics: Life Cycle Assessment. In *Perovskite Photovoltaics, Academic Press*. pp 447-480.

Nielsen, UN; Wall, DH; Six, J (2015). Soil biodiversity and the environment. *Annu. Rev. Environ. Resources.* 40:63–90.

Nzili, A. (2018). Current status of the degradation of aliphatic and aromatic petroleum hydrocarbons by thermophilic microbes and future perspectives. *Int. J. Environ. Res. Public Health*. 15(12): 2782.

Obire, O; Anyanwu, EC; Okigbo, RN (2008). Impact of various concentrations of crude oil on the physicochemical properties of a soil in Nigeria. *Environ. Res. J.* 2 (2/3):249-258.

Oertel, C; Matschullat, J; Zurba, K; Zimmermann, F; Erasmi, S (2016). Greenhouse gas emissions from soils—A review. *Geochemistry*. 76:327-352.

Omotayo, AE; Ojo, OY; Amund, OO (2012). Crude oil degradation by microorganisms in soil composts. *Res. J. Microb.* 7(4):209-218.

Pathak, H., Bhatnagar, K; Jaroli, D. P. (2011). Physico-chemical properties of petroleum polluted soil collected from Transport Nagar (Jaipur). *Indian J. Fundam. Appl. Life Sci.* 1(3): 84-89.

Saadoun, I; Mohammad, MJ; Hameed, KM; Shawaqfah, MA (2008). Microbial populations of crude oil spill polluted soils at the Jordan-Iraq desert (the Badia region). *Brazilian J. Microbiology*. 39(3): 453-456.

Schoonover, JE; Crim, JF (2015). An introduction to soil concepts and the role of soils in watershed management. *J. Contemp. Wat. Res. Educ*. 154:21–47.

Silver, WL; Perez, T; Mayer, A; Jones, AR (2021). The role of soil in the contribution of food and feed. Phil. Trans. R. Soc. B. 376: 3.