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Preliminary Study of Crude Oil Degradation by Microorganisms Isolated from Polluted Soil in Okarki, River State, Nigeria

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Abstract Pollution of the environment by hydrocarbon compounds has become a significant management challenge in oil-producing countries. Presently, the use of biological means for the reclamation of polluted sites is the most acceptable technique owing to its eco-friendliness. This study was carried out to assess the crude oil degradation potential of indigenous microorganisms in polluted soil. The polluted soil was obtained from a crude oil-ladened site in Okarki, River State, Nigeria. Bacterial and fungal organisms present in the polluted soil were isolated on Nutrient agar and Saboraud dextrose agar plates respectively. The isolates were identified based on their morphological, microscopic and biochemical characteristics. Gravimetric analysis of the crude oil degradation by the isolates was done in Bushnell Hass medium supplemented with 5% crude oil as the only carbon source. A total of 6 bacterial genera namely, *Staphylococcus, Citrobacter, Micrococcus, Pseudomonas, Bacillus* and *Corynebacterium* were identified while the fungal isolates were *Aspergillus niger, Aspergillus flavus* and *Penicillium* sp. Bacterial and fungal counts were 2.57±0.01 Log cfu/g and 2.08±0.07 Log cfu/g respectively. *Bacillus* sp. had the highest relative abundance (27.3%), while *Micrococcus* sp. and *Corynebacterium* sp. had the least occurrence (9.1%). Among the fungal group, *A. niger* showed the highest percentage occurrence in the polluted soil. All the indigenous organisms isolated from the polluted soil showed varying potentials for crude oil degradation. *Bacillus* sp. and *Penicillium* sp. were the highest crude oil-degrading bacterium and fungus respectively. The degradation potential of the bacterial consortium was significantly higher ($P < 0.05$) than the other consortia tested. This study has shown that indigenous organisms possess the potential for crude oil degradation.

Keywords: *crude oil, degradation, bacteria consortium, reclamation, fungi consortium*

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1. Introduction

The shift in economic base of coal to crude oil and petroleum products, significantly escalated the quantity of these commodities being shipped across the high seas. This, coupled with their storage underground, involve high environmental risks [1]. As a result of contamination from petrochemical products and industrial effluents, diverse components of crude oil and petroleum such as polycyclic aromatic hydrocarbons (PAHs) have been found in waterways [2].

Petroleum hydrocarbon pollution of the environment may also arise from oil well drilling production operations, transportation and storage in the upstream industry, marketing in the downstream industry, and intentional bunkering of pipeline [3]. Other sources of petroleum and its products in the environment also include accidental spills and ruptured oil pipelines, as pipelines are vulnerable to ‘’tear and wear’’, thus can fail with time [4].

The spilled petroleum hydrocarbons seeps into the soil due to gravity until an impervious horizon is met, for example bedrock, watertight clay or an aquifer. Poor miscibility of crude oil accounts for accumulation of free oil on the surface of ground water and this may drift over a wide distance to contaminate other zones very far away from the point of pollution [5].

Oil suffocates the soil particles, prevents ease of air diffusion in the soil pores, and creates an anaerobic environment which affects soil microbial communities [6]. Dense crude oil pollution can cause complete death of marsh vegetation [7]. In addition, crude oil-contaminated soils are hydrophobic compared with pristine sites [8]. Hydrocarbon contamination can also increase soil total organic carbon [9], and change soil pH values [10], and other soil chemical properties [11]. Crude oil affects soil fertility, germination and growth of some plants; however, the severity of the impact depends on the quantity and type of oil spilled [7]. Pollution of environment affects humans exposed to it. Some health conditions have been associated with exposure to crude oil pollution [12]. The

health problems may be through any or combinations of the following routes: contaminated food and / or water, emission and / or vapors [1]. The volatile organic components of crude oil have been implicated in the aggravation of asthma, bronchitis and accelerated aging of the lungs. They also affect the liver, kidney and spleen [12]. Epidemiological evidence suggests that oil spills affect neonates, contributes to infant mortality, and also increase the risk of abortion and stillbirth [13].

Oil spills are common occurrences in Niger Delta region of Nigeria, where over 40 million liters of crude oil spill have been recorded annually, resulting in human deaths and damage to the local ecosystem. It has been reported that more than 12,000 oil spill incidents have occurred in the oil-rich region between 1976 and 2014 [14].

Bioremediation recently has evolved as an emerging green technology of environmental conservation by removing, transforming and breaking down various contaminants, especially petroleum hydrocarbons, by applying living organisms [15]. Degradation can occur aerobically or anaerobically, however, greater percentage of hydrocarbon degradation occur under aerobic condition. This process uses microbial metabolism in the presence of optimum environmental conditions and sufficient nutrients to breakdown contaminants notably petroleum hydrocarbons [16]. Bioremediation requires the evaluation of both the intrinsic degradation capacities of the autochthonous microflora and the environmental parameters involved in the kinetics of the *in-situ* process [17].

Autochthonous microorganisms, which are the indigenous microorganisms living on polluted environment are usually well adapted to the presence of the pollutants, as a result of long-term exposure to site-specific stress factors [18]. The adaptation provides useful opportunities for bioremediation at such sites [19]. Therefore, several studies have focused on the community of microorganisms associated with oil-contaminated soil, and assessment of their bioremediation potentials [20,21,22]. Bacteria and fungi have been reported as the principal agents of hydrocarbon degradation [23]. Several genera have been reported as hydrocarbon degraders [24], and they include *Achromobacter*, *Pseudomonas*, *Acinetobacter*, *Alkanindiges*, *Alter-omonas*, *Arthrobacter*, *Burkholderia*, *Dietzia*, *Enterobact-er*, *Kocuria*, *Marinobacter*, *Mycobacterium*,

Pandoraea, Bacillus, Staphylococcus, Streptobacillus, Streptococcus, Rhodococcus and Micrococcus [25].

The aim of the present study is to assess the crude oil degradation potential of autochthonous microorganisms in a polluted soil.

2. Materials and Methods

2.1. Description of Okarki Sampling Site

Okarki communitiy in Ahoada East Local Government Area of River State, is a border town between Bayelsa and River state. It is located on latitude: 4˚58’56”N and longitude 6˚25’44”N. The sampling site is one of the sites of illegal refinery of crude oil [26].

2.2. Sample Collection

Soil samples contaminated with crude oil were aseptically collected by random sampling technique at 6cm depth in the sampling site. The soil samples were collected from 6 different points in the site, mixed and homogenized to obtain a composite sample.

2.3. Isolation of Indigenous Microorganisms

Indigenous microorganisms in the polluted soil samples were isolated and enumerated using standard pour plate method [26]. 1.0g of the composite soil was serially diluted tenfold and 1ml of 10^{-2} dilution inoculated on Nutrient agar (Oxoid) plate. The plate was observed for bacterial growth after 24h incubation at 30°C. Triplicate plates were prepared. Developed colonies were counted and recorded as mean heterotrophic bacteria count ± standard deviation. The colonies were sub-cultured and pure cultures obtained were stored on Nutrient agar slants at 4^0C for further studies.

The soil sample was similarly treated for the isolation of fungi. 1ml of 10^{-2} dilution was inoculated on Saboraud dextrose agar (SDA) plate containing 0.05g/ml of chloramphenicol, and the plate incubated for 72h at 30°C. Fungal count was recorded and pure cultures obtained stored on SDA slant at 4°C for further studies.

2.4. Identification of Bacterial Isolates

Several biochemical tests were carried out to identify the bacterial isolates from the polluted soil sample following the method described by [27]. They include Gram reaction, catalase test, coagulase test, motility test, indole test, methyl red test, Voges-Proskauer test, citrate utilization test, sugar fermentation test.

2.5. Identification of Fungal Isolates

The fungal isolates were identified based on detailed studies of their colonial morphologies and microscopic features, and compared to standard description given by [28,29].

2.6. Shake Flask Experiment of Crude Oil Degradation by Indigenous Microorganisms

2.6.1. Preparation of Inoculum

A 24h old culture of the isolate was inoculated into 10ml of sterile distilled water in a test tube and standardized using 0.5 Mcfarland standard [30]. This served as the seed inoculum

2.6.2. Biodegradation Assay

Shake flask experiment was carried out to assess the crude oil degrading potential of the isolated indigenous microorganisms [31]. 1ml of seed inoculum of the isolate was inoculated into 50ml of Bushnell-Haas broth supplemented with 5.0% w/v crude oil in 250ml Erlenmeyer flask. The flask was incubated at 30°C on a rotary shaker at 150rpm. An un-inoculated medium served

as control. After 7 days incubation, residual crude oil was extracted and crude oil degradation rate was estimated gravimetrically.

2.6.3. Extraction Process

To the culture broth was added 5ml of n-hexane and the flask contents mixed thoroughly before transferring to a separating funnel to extract the residual crude oil [31]. Extraction process was carried out twice to ensure complete recovery of oil. The extract was treated with 0.4g anhydrous sodium sulphate to remove the moisture, and the suspension decanted into a pre-weighed beaker, leaving behind sodium sulphate. The pre-treated extract was evaporated to dryness by heating in a water bath. The weight of the extracted crude oil was deducted from the previously weighed beaker.

The % degradation of the crude oil was calculated as:

Weight of residual crude oil= Weight of beaker containing extracted crude oil - Weight of empty beaker.

Amount of crude oil degraded = Weight of crude oil added in the media - Weight of residual crude oil

% degradation = (Amount of crude oil degraded / Amount of crude oil added in the media) x 100.

2.7. Statistical Analysis

Data obtained was subjected to one way Analysis of Variance by Student-Newman-Keul (SNK) test at 95% confidence level using IBM SPSS statistics version 20 [32].

3. Results and Discussion

3.1. Isolation of Indigenous Microorganisms

The results obtained in this study show the presence of different genera of bacteria and fungi in the crude oil-contaminated soil (Table 1 - Table 2). The mean heterotrophic bacterial count in the polluted soil sample was 2.57±0.01 Log Cfu/g. This is in line with the observation made by [33], in crude oil-contaminated soil in Port Harcourt, River State. However, our finding does not corroborate the report of [34], who recorded high bacterial count (5.903CFU/g - 9.69CFU/g) in oil-saturated desert soil samples. In another study, bacterial populations of crude petroleum-polluted soil counts ranged from 5.18 to 7.38CFU/g soil at a soil depth of 1-10 cm [23]. The reduced bacterial count observed in this study could be attributed to the long term impact of the crude oil pollution on the abundance of microbial communities in the site.

The mean fungal count obtained in this study was 2.08±0.07 LogCFU/g. The range of heterotrophic fungal count obtained in this study supports that recorded by [35]. A significantly higher fungal count (p value < 0.05) was obtained in crude oil-polluted soil by [36].

The bacterial count in this study was significantly higher (p value < 0.05) than the fungal count. It is very likely that bacterial organisms were more adapted to the polluted soil than the fungi, hence were able to multiply and increase in population. In a study by [37], on the response of bacterial and fungal communities to high petroleum pollution in different soils, the bacterial communities were significantly higher than the fungal communities.

3.2. Identification of Bacterial Isolates

The characteristic features of the bacterial isolates from the polluted soil are shown in Table 1.

A total of six indigenous bacteria was identified from the crude oil-polluted soil and they belong to the genera *Staphylococcus*, *Citrobacter*, *Micrococcus*, *Pseudomonas*, *Bacillus* and *Corynebacterium* (Table 1). These bacterial isolates make the list of the commonly isolated microorganisms from hydrocarbon-polluted environment, and this observation supports the work of [35]. This finding is also in agreement with the work of [38], who isolated nine different genera of bacteria including *Staphylococcus*, *Micrococcus*, *Bacillus*, *Pseudomonas*, *Acinetobacter*, *Enterobacter*, *Escherichia*, *Klebsiella* and *Proteus* from hydrocarbon-polluted soil in Effurun, Delta State. Similarly, *Bacillus* spp., *Pseudomonas* spp., *Micrococcus* spp., *Serratia* spp., *Arthrobacter* spp., *Proteus* spp. and *Shigella* spp. were isolated from three different crude oil-polluted sites in Anambra state [39]. *Bacillus* and *Pseudomonas* spp. were recovered in a long-standing petroleum-contaminated sediments in Bohai Bay, China [40].

The relative abundance of the bacterial isolates as presented in Figure 1, shows that *Bacillus subtilis* was the predominant species (27.3%) while *Micrococcus* sp. and *Corynebacterium* sp. were the least occurring bacteria. Contrary to our findings, various researches showed that *Pseudomonas* spp. are predominantly detected in diverse hydrocarbon-polluted environment [34,41,42].

3.3. Identification of Fungal Isolates

The morphological and microscopic features of the fungal organisms are presented in Table 2.

The fungal isolates are *Aspergillus niger*, *Aspergillus flavus* and *Penicillium* (Table 2). This finding supports the work of [43] in which *Penicillium* spp., *Aspergillus niger* and *Candida* sp., *Mucor* sp., *Rhodotorulla* sp, *Rhizopus* sp, *Trichorderma* sp. and *Cladospermorium* sp. were recovered from a crude oil-polluted soil in Bayelsa state, Nigeria. Our inability to isolate the other fungi may been as a result of the medium used. In a study by [44] on the response of fungi to diesel contamination, *Aspergillus niger*, *Aspergillus* spp., *Fusarium* sp., *Mucor* sp., *Rhizopus* sp. and *Saccharomyces* sp. were isolated**.**

The dominant fungus recovered in this study was *Aspergillus niger* (Figure 1), having 60% relative abundance. This result corroborates the report of [45], who recorded *Aspergillus niger*, and *Fusarium solani* as the predominant fungi in a petroleum hydrocarbon polluted soil. However, [46] recorded *Aspergillus oryzae* and *Mucor irregularis* as the most abundant fungi in a crude oil-contaminated field with 56.67% and 66.70% abundances respectively.

Table 1. Characteristic features of the indigenous bacterial isolates from polluted soil

Biochemical/morphological tests	Isolates					
	S.aureus	***Micrococcus***	***P.aeruginosa***	***Citrobacter***	***Bacillus***	***Corynebacterium***
Gram reaction	+ cocci	+ cocci	- rod	- rod	+ rod	+ rod
Motility	-	-	+	+	-	+
Citrate	+	+	+	+	-	+
Indole	-	+	-	-	-	-
Coagulase	+	-	-	-	-	+
Catalase	+	+	+	+	+	+
Oxidase	-	+	+	-	-	-
Methyl Red	+	-	-	+	+	-
Voges Proskauer	-	+	-	-	-	+
Sugar fermentation						
Sucrose	+	+	-	+	-	+
Maltose	-	+	-	+	+	+
Glucose	-	+	-	+	+	+
Lactose	-	-	-	+	-	+
Rhamnose	-	+	+	+	+	-

Key: - = negative, + = positive.

Table 2. Morphological and microscopic features of fungal isolates from polluted soil

Isolate	Macroscopic features	Microscopic features
Penicillium sp.	Greenish rough colonies with white edges on the front and milky colour on the reverse	Septate hyphae, branching conidiophore stipes with clublike philaides and conidia arranged in chains
Aspergillus niger	Round brownish dark cottony colony with white edges on the front and milky on the reverse	Septate hyhae, variable length and smooth conidiophore, vesicles end in metulae which gives rise to the phialides with globular conidia
Aspergillus flavus	Yellowish green cottony colonies on the surface and brown on the reverse	Septate hyphae, smooth conidiophore with round head. Conidia are clustered on the phialide head

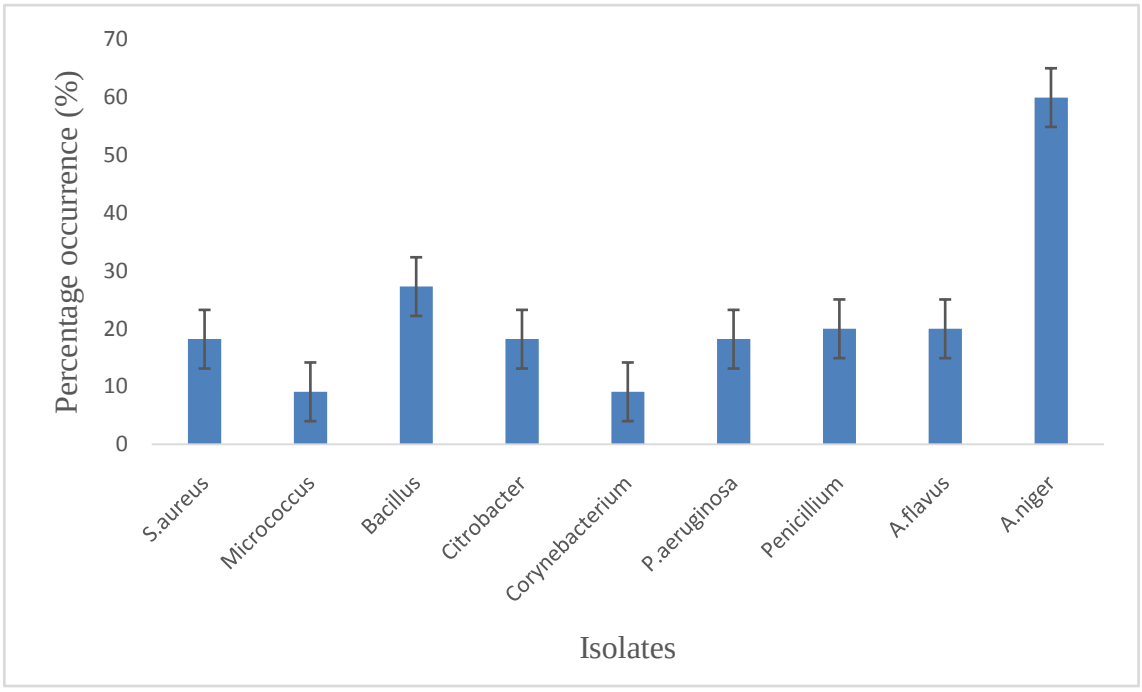

Figure 1. Percentage occurrence of bacterial and fungal isolates in the polluted soil

3.4. Shake Flask Experiment of Crude Oil Degradation by Indigenous Microorganisms

Results of crude oil degradation by the indigenous microorganisms isolated from the crude oil-polluted soil presented in Figure 2, show that all the bacterial and fungal isolates had the ability to degrade crude oil. Crude oil degradation rate ranged from 6.7± 2.3% to 98.8 ± 0.7%, with *Bacillus subtilis* achieving the highest degradation of crude oil, while *Micrococcus* sp. was the least. There was no significant difference (p-value > 0.05) in the degradation rates of *S.aureus*, *P.aeruginosa*, *B. subtilis* and bacteria consortium, indicating that they had the same level of performance. Some microorganisms have been reported to have inherent ability to degrade hydrocarbon, and probably their enzyme system aid them in the breakdown of different classes of hydrocarbons [47,48,49]. It has been reported that some organisms especially *P.aeruginosa* are capable of synthesizing different classes

of biosurfactants in the presence of hydrophobic substrate, which makes the hydrocarbon bioavailable to them [50]. *Pseudomonas* sp. had high crude oil degradation rate (97.1%) in this study (Figure 2). Similar high crude oil degradation rate (93%) with *Bacillus cereus* was reported by [51], during their work on biodegradation of crude oil hydrocarbons by a newly isolated biosurfactant producing strain. *Pseudomonas* sp achieved 95% crude oil degradation and was reported as the most efficient crude oil degrader in Coastal area of Yanbu, Saudi Arabia by [52].

The optimal degradation of crude oil achieved by the bacteria consortium could be as a result of synergistic interaction among the bacterial organisms. This observation is in line with the report of [40], who recorded 80.4% crude oil degradation rate with mixed bacteria culture.

The crude oil degradation potential of the bacteria consortium was significantly higher (p-value > 0.05) than the consortium of the fungal isolates (Figure 2). [53], opined that bacteria, single species or consortium, are more efficient hydrocarbon degraders than other microorganisms. In a study carried out by [54], hydrocarbon degradation rates of 10.68 -15.67% was recorded by single bacterial strains, however, the synergistic effect of the bacterial strains in consortium produced higher total petroleum hydrocarbon degradation (19.59%).

The degradation rate (56%) of crude oil observed with *Penicillium* sp. (Figure 2) in this study is in line with the report of [55]. They noted a 57% and 55% crude oil degradation by *Penicillium* sp. RMA1 and *Penicillium* sp. RMA2 respectively from isolates recovered in Rumaila oil field, River State. In contrast to our findings, *Aspergillus niger* was reported as the highest crude oil-degrader against other isolates (*Candida glabrata*, *Candida krusei* and *Saccharomyces cerevisiae*) recovered in Basrah refinery field, Iraq [56].

The fungi consortium as presented in Figure 2, also achieved a degradation rate of 46.7±6.1% when compared to single fungal isolates. However, high degradation of petroleum hydrocarbon (92%) with fungi consortium comprising of *Aspergillus terreus*-SRF-15, *Fusarium proliferatum*-SRF-50, *Fusarium* sp-SRF-58 and *Aspergillus* sp-SRF-67 was reported by [57].

The poor degradation of crude oil (Figure 1) with bacteria and fungi consortium and *Aspergillus* spp. observed in this study is contrary to the report of [58]. They recorded a 42.24% crude oil degradation by bacteria and fungi consortium and 47.72% by *Aspergillus fumigatus* in their comparative study of petroleum crude oil degradation potential of microbes from petroleum-contaminated soil and non-contaminated soil.

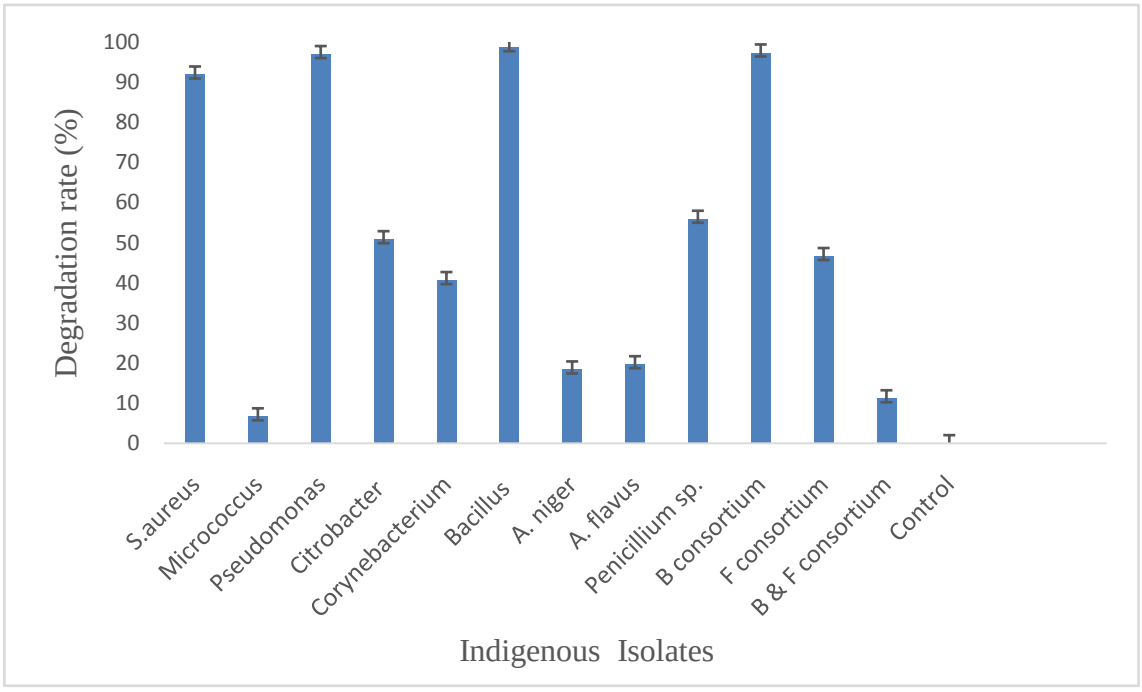

Figure 2. Crude oil degradation by indigenous organisms (Key: B = bacteria; F = fungi)

4. Conclusion

The ability to remove crude oil from soil by indigenous microorganisms was observed in this study. *Bacillus* sp., *Staphylococcus aureus*, *Pseudomonas* sp. and the bacteria consortium achieved above 90% crude oil degradation. This shows that natural attenuation can be employed for reclamation of crude oil-polluted soil. Biodegradation is a complex process, which is influenced by several environmental factors, therefore, further research is necessary to improve the degradation potentials of the indigenous organisms.

Competing Interest

The authors have no competing interests.

References

[1] Onwurah, I. N. E., Ogugua, V. N., Onyike, N. B., Ochonogor, A. E. and Otitoju, O. F. "Crude oil spills in the environment, effects and some innovative clean-up biotechnologies", *International Journal of Environmental Research*, 1(4). 307-320. July 2007.

[2] Lawal, A.T. "Polycyclic aromatic hydrocarbons: A review", *Cogent Environmental Science*, 3(1). July 2017.

[3] Liu, X, *Advantages of Multidimensional Chemical Fingerprinting in Identifying the Source of Marine Oil Spills in Bohai Bay, China. In: Oil spill environmental forensics case studies*, Butterworth-Heinemann, 2018, 239-253.

[4] Baird, J. "Oil's shame in Africa", Newsweek. Published on July 26, 2010. 27.

[5] Wang, Y., Feng, J., Lin, Q., Lyu, X., Wang, X. and Wang, G, "Effects of crude oil contamination on soil physical and chemical properties in Momoge wetland of China", *Chinese Geographical Science*, 23(6). 708-715. November 2013.

[6] Sutton, N. B., Maphosa, F. and Morillo, J. A, "Impact of long-term diesel contamination on soil microbial community structure", *Applied and Environmental Microbiology*, 79(2). 619-630. November 2012.

[7] Lin, Q. and Mendelssohn, I. A, "Impacts and recovery of the deep-water horizon oil spill on vegetative structure and function of coastal salt marsh in the Northern Gulf of Mexico" *Environmental Science and Technology*, 46(7). 3737-3743. March 2012.

[8] Aislabie, J. M., Balks, M.R. and Foght, J. M, "Hydrocarbon spills on antarctic soils: effects and management", *Environmental Science and Technology*, 38(5). 1265-1274. January 2004.

[9] Ikhajiagbe, B. and Anoliefo, G. O, "Impact of soil amendment on phytotoxicity of a 5-month old waste engine oil polluted soil", *African Journal of Environmental Science and Technology*, 4(4). 215-225. January 2010.

[10] Wang, X. Y., Feng, J., Zhao, J. M, "Effects of crude oil residuals on soil chemical properties in oil sites, Momoge Wetland, China" *Chinese Geographical Science*, 23(6). 708-715. November 2013.

[11] Kisic, I., Mesic, S. and Basic, F, "The effect of drilling fluids and crude oil on some chemical characteristics of soil and crops", *Geoderma*, 149(3-4), 209-216. 2009.

[12] D'Andrea, M.A. and Reddy, G.K, "Health risks associated with crude oil spill exposure", *American Journal of Medicine*, 127(9). 886 -899. May 2014.

[13] Bruederle, A. and Hodler, R, "Effect of oil spills on infant mortality in Nigeria". *Proceedings of the National Academy of Sciences of the United States of America*, 116 (12). 5467-5471. March 2019.

[14] Adebayo, B, "Major new inquiry into oil spills in Nigeria's Niger Delta launched". CNN. Updated 0018 GMT (0818 HKT) on March 27, 2019.

[15] Mahmoud, Y.A.G, "Advancement in bioremediation process: A mini review" *International Journal of Environmental and Technological Sciences*, 3. 83-94. 2016.

[16] Adams, G.O., Fufeyin, P.T., Okoro, S.E. and Ehinomen, I, "Bioremediation, biostimulation and bioaugmention: A Review" *International Journal of Environmental Bioremediation and Biodegradation*, 3(1). 28-39. March 2015.

[17] Alkorta, I., Epelde, L. and Garbisu, C., "Environmental parameters altered by climate change affect the activity of soil microorganisms involved in bioremediation", *FEMS Microbiology Letters*, 364(19). 1-7. September 2017.

[18] Touceda-González, M., Prieto-Fernández, Á., Renella, G., Giagnoni, L., Sessitsch, A., Brader, G., Kumpiene, J., Dimitriou, I., Eriksson, J., Friesl-Hanl, W., Galazka, R., Jamssem, J., Mench, M., Muller, I., Neu, S., Puschenreiter, M., Siebielec, G., Vangronsveld, J. and Kidd, P.S, "Microbial community structure and activity in trace element-contaminated soils phyto-managed by Gentle Remediation Options (GRO)" Environtal Pollution, 231. 237-251. December 2017.

[19] Oliveira, T., Mucha, A.P., Reis, I., Rodrigues, P., Gomes, C.R. and Almeida, C.M.R, "Copper phytoremediation by a salt marsh plant (*Phragmites australis*) enhanced by autochthonous bioaugmentation" *Marine Pollution Bulletin*, 88. 231-238. November 2014.

[20] Leys, N.M., Ryngaert, A., Bastiaens, L., Verstraete, W., Top, E.M. and Springael, D, "Occurrence and phylogenetic diversity of *Sphingomonas* strains in soils contaminated with polycyclic aromatic hydrocarbons" *Applied Environmental Microbiology*, 70. 1944-1955. April, 2004.

[21] Viñas, M., Sabaté, J., Espuny, M.J. and Solanas, A.M, "Bacterial community dynamics and polycyclic aromatic hydrocarbon degradation during bioremediation of heavily creosote-contaminated soil" *Applied and Environmental Microbiology*, 71. 7008-7018. November 2005.

[22] Pizarro-Tobías, P. Niqui, J.L., Roca, A. Solano, J., Fernández, M., Bastida, F., García, C. and Ramos, J.L. "Field trial on removal of petroleum-hydrocarbon pollutants using a microbial consortium for bioremediation and rhizoremediation." *Environmental Microbiology Report*, 7(1). 85-94. February 2015.

[23] Saadoun, I., Mohammad, M.J., Hamed, K.M. and Shawaqfah, M, Microbial populations of crude oil spill polluted soils at the Jordan-Iraq desert (the Badia region). *Brazilian Journal of Microbiology*, 39(3). 453-456. July 2008.

[24] Tremblay, J., Yergeau, E., Fortin, N., Cobanli, S., Elias, M. and King, T. L, "Chemical dispersants enhance the activity of oil-and gas condensate-degrading marine bacteria" *International Society of Microbial Ecology Journal*, 11. 2793-2808. August 2017.

[25] Xu, X., Liu, W., Tian, S., Wang, W., Qi, Q., Jiang, P., Gao, X., Li, F., Li, H. and Yu, H, "Petroleum hydrocarbon-degrading bacteria for the remediation of oil pollution under aerobic conditions: A perspective analysis" *Frontiers in Microbiology*, 9. 2885. December 2018.

[26] Atlas, R.M, *Alphabetical listing of media. Handbook of microbiological media for the examination of food*, Taylor and Francis group, London. 1995, 12.

[27] Cheesbrough, M, *Microbiological test. In: District laboratory practice in tropical countries*. Cremer, A. and Evan, G. edition. Cambridge University Press, UK. 2000, 1-226.

[28] Nadia, E.A., Ibtissam, B.S., Mohamed, B.K., Mahmoud, M. and Naima, B, "Isolation and identification of fungal communities in organic and conventional soils" *International Journal of Current Microbiology and Applied Sciences*, 6(4). 1111-1123. April 2017.

[29] Diba, K., Kordbacheh, P., Mirhendi, S.H., Rezaie, S. and Mahmoud, M, "Identification of *Aspergillus* species using morphological characteristics" *Pakistan Journal of Medical Sciences*, 23(6). 867 - 872. October -December 2007.

[30] Patel, J.B., Cockerill, F. R., Bradford, P.A., Eliopoulos, G.M., Hindler, J.A., Jenkins, S.G., Lewis, J.S., Limbago, B., Miller, L.A., Nicolau, D.P., Powell, M., Swenson, J.M., Traczewski, M.M., Turnidge, J.D., Weinstein, M.P. and Zimmer, B.L, "Performance standards for antimicrobial susceptibility testing; twenty-fifth informational supplement (M100-S25)" Clinical and Laboratory Standard Institute, Wayne, Pennsylvania. 2015, 132-135.

[31] Anaukwu, C.G., Ezemba, C.C., Anakwenze, V.N., Agu, K.C., Okeke, B.C., Awah, N.S. and Ekwealor, I.A, "Effect of Biosurfactant Produced by *Citrobacter murliniae* AF025369 and a Synthetic Surfactant on Degradation of crude Oil", *Edorium Journal of Microbiology*, 2. 1-6. February 2016.

[32] Garth, A. "Analysing data using SPSS." https://students.shu.sc.uk/lits/it/documents/pdf/analysing_data_using_spss.pdf. 2018. Assessed on 20/3/2019.

[33] Ogbonna, D.N., Douglas, S.I. and Awari, V.G, "Characterization of hydrocarbon utilizing bacteria and fungi associated with crude oil contaminated soil", *Microbiology Research Journal International*, 30(5). 54-69. June 2020.

[34] Ali, N., Dashti, N., Khanafer, M., Al-Awadhi, H. and Radwan, S, "Bioremediation of soils saturated with crude oil" *Scientific Reports*, 10. 1116. January 2020.

[35] Onifade, A. K and Abubakar, F.A, "Characterization of hydrocarbon-degrading microorganisms isolated from crude oil contaminated soil and remediation of the soil by enhanced natural attenuation" *Research Journal of Microbiology*, 2(2). 149-155. 2007.

[36] Olukunle, O. F, "Characterization of indigenous microorganisms associated with crude oil-polluted soils and water using traditional techniques", *Microbiology Journal*, 3(1), 1-11. July 2013.

[37] Galitskaya, P., Biktasheva, L., Blagodatsky, S. and Selivanovskaya, S, "Response of bacterial and fungal communities to high petroleum pollution in different soils" *Scientific Reports*, 11. 164. January 2021.

[38] Ataikiru, T.L., Okorhi-Damisa, B.F. and Akpaiboh, J.I, "Microbial community structure of an oil polluted site in Effurun, Nigeria" *International Research Journal of Public and Environmental Health*, 4 (3). 41-47. April 2017.

[39] Umeaku, C. N., Emmy-Egbe, I. O., Ukoha, C. C., Ezenwa, S. E. and Chris-Umeaku, C. I, "Biodegradation of crude oil-polluted soil by bacterial isolates from Nigeria", *Frontiers in Environmental Microbiology*, 5 (1). 14-28. March 2019.

[40] Tian, X., Wang, X., Peng, S., Wang, Z., Zhou, R. and Tian, H, "Isolation, screening, and crude oil degradation characteristics of hydrocarbons-degrading bacteria for treatment of oily wastewater"

Water Science and Technology, 78(12). 2626-2638. December 2018.

[41] Egbo, W.M., Onyewuchi, A. and Gideon, A, "Screening of hydrocarbon degrading fungi in crude oil-polluted soil obtained in the Niger Delta" African Journal of Environmental Science and Technology, 12(5). 172 - 176. May 2018

[42] Wemedo, S.A. and Ekine, P.T, "Response of fungi to diesel oil-contamination of a soil in Nigeria" International Journal of Microbiology, Genetics and Molecular Biology Research, 3(2). 21-27. December 2017.

[43] Teknikio, J. B., Adeyemo, J. A., Ojeniyi, S. O. and Tate, J. O, Isolation and identification of bacteria in petroleum hydrocarbons polluted soils in North-West, Bayelsa State", *Covenant Journal of Physical and Life Sciences*, 1(2). 1-13. September 2018.

[44] Bhattacharya, D., Sarma, P.M., Krishnan, S., Mishra, S., and Lal, B, "Evaluation of genetic diversity among *Pseudomonas citronellolis* strains isolated from oily sludge-contaminated sites" *Applied and Environmental Microbiology*, 69. 1435-1441. March 2003.

[45] Al-Jawhari, I.F.H, "Ability of some fungi in biodegradation of petroleum hydrocarbon" Journal of Applied and Environmental Microbiology, 1292). 46-52.

[46] Asemeloye, M.D., Tosi, S., Dacco, C., Wang, X., Xu, S., Marchisio, M.A.Gao, W., Jonathan, S.G. and Pecoraro, L, "Hydrocarbon degradation and enzyme activities of *Aspergillus oryzae* and *Mucor irregularis* isolated from Nigerian crude oil-polluted sites" *Microorganisms*, 8.1912-1931. November 2020.

[47] Nurul, H. M. B. H, Mohamad, F. I, Norhayati, R. and Suraini, A, "Production of biosurfactant produced from used cooking oil by *Bacillus* sp. HIP3 for heavy metals removal" *Molecules*, 24(14).2617. July 2019.

[48] Cheng, T., Liang, J., He, J., Hu, X., Ge, Z. and Liu, J, "A novel rhamnolipid-producing-*Pseudomonas aeruginosa* ZS1 isolate derived from petroleum sludge suitable for bioremediation", *Applied Microbiology and Biotechnology Express*, 7. 120. June 2017.

[49] Eddouaouda, K., Mnif, S., Badis, A., Younes, S.B., Cherif, S., Ferhat, S., Mhiri, N., Chamkha, M. and Sayadi, S, "Characterization of a novel biosurfactant produced by *Staphylococcus* sp. strain 1E with potential application on hydrocarbon bioremediation", *Journal of Basic Microbiology*, 51. 1-11. November 2011.

[50] Anaukwu, C.G., Ogbukagu, C.M. and Ekwealor, I.A, "Optimized biosurfactant production by *Pseudomonas aeruginosa* strain CGA1 using agro-industrial waste as sole carbon source", *Advances in Microbiology*, 10. 543-562. October 2020.

[51] Christova, N., Kabaivanova, L., Nacheva, L., Petrov, P. and Stoineva, I, "Biodegradation of crude oil hydrocarbons by a newly isolated biosurfactant producing strain", *Journal of Biotechnology and Biotechnological Equipment*, 33(1). 863-872. May 2019.

[52] EL-Hanafy, A,M., Anwar, Y., Mohamed, S.A., Al-Garni, S.M.S., Sabir, J.S.M., AbuZinadah, O.A., Al Mehdar, H., Alfaidi, A.W. and Ahmed, M.M.M, "Isolation and identification of bacterial consortia responsible for degrading oil spills from the coastal area of Yanbu, Saudi Arabia" *Biotechnology and Biotechnological Equipment*, 30(1). 69-74. October, 2015.

[53] Abdulla, K.J., Ali, S.A., Gatea, I.H., Hameed, N.A. and Maied, S.K, "Biodegradation of crude oil using local bacterial isolates" *IOP Conference series: Earth and Environmental Science*, 388. 012081.

[54] Ghorbannezhad, H., Moghimi, H. and Dastgheib, S. M. M, "Evaluation of heavy petroleum degradation using bacterial-fungal mixed cultures" *Ecotoxicology and Environmental Safety*, 164. 434-439. November 2018.

[55] Al-Hawash, A.B., Alkooranee, J.T., Abbood, H.A., Zhang, J., Sun, J., Zhang, X. and Ma, F, "Isolation and characterization of two crude oil-degrading fungi strains from Rumaila oil field", *Iraq Biotechnology Reports*, 17.104-109. March 2018.

[56] Burghal, A. A., Abu-Mejdad, N. M.J. and Al-Tamimi, W. H, "Mycodegradation of crude oil by fungal species isolated from petroleum contaminated soil", *International Journal of Innovative Science Engineering and Technology*, 5. 1517-1524. February 2016.

[57] Ramoutar, S. Mohammed, A. and Ramsubhag, A, "Laboratory-scale bioremediation potential of single and consortia fungal isolates from two natural hydrocarbon seepages in Trinidad, Indies", *Bioremediation Journal*, 23(3). 131-141. August 2019.

[58] Ra, T., Zhao, Y. and Zheng, M, "Comparative study on the petroleum crude oil degradation potential of microbes from petroleum-contaminated soil and non-contaminated soil", International Journal of Environmental Science and Technology, 2. 11. November 2018.