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EVALUATION OF THE EFFICIENCY OF INTRINSIC MICROORGANISMS IN THE REMEDIATION OF CRUDE OIL POLLUTED SOIL MICROCOSMS

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Abstract
Soil provides habitat for diverse types of microorganisms. This study investigated the effectiveness of indigenous soil microorganisms in natural attenuation. Soil samples used in this study were collected from Awoye, Orioke- Iwamimo, Igodan- Lisa, Oba-Ile and Idoani all in Ondo State, Nigeria. Samples were collected into sterile black cellophane bags using hand auger at depth of 15- 20 cm. One set (200 g) of partially air- dried, homogenized and sieved samples were sterilized by autoclaving for three consecutive times at 121°C for 30 min. The moisture contents of the soil samples were adjusted with sterile water to obtain 25% (w/w) and contaminated with sterile crude oil by thoroughly mixing crude oil with the soils to obtain 5% (w/w) crude oil pollution. The weight loss method was used to determine the amount of crude oil in samples and then evaluated for amount of crude oil lost at intervals of 30 days for six months. The results indicated that the amount of crude oil degraded was higher in non- sterile soil samples (41.01-49.00%) than the sterile samples (17.00 -22.99%). This implies that crude oil degradation by intrinsic soil microorganisms constituted 52.33-58.74% of crude oil reduction by natural attenuation. It could be concluded that crude oil removal from soil occurred without human intervention due to the activities of autocthonous microorganisms. This study concludes that soil is rich in microorganisms possessing the desired catabolic potentials for crude oil utilization.

Key words: Indigenous soil microorganisms, Crude oil, Oil spillage, Pollution, Remediation, Natural attenuation, Bioremediation.

Introduction

Crude oil is an extremely complex mixture of different naturally occurring hydrocarbons, liquid in their natural state is mainly composed of paraffins, naphthenes, aromatics, and asphaltics. The hydrocarbons are primarily alkanes, cycloalkanes and aromatic hydrocarbons (Agarry *et al.*, 2012; Cho *et al.*, 2012, Sinnathambi and Nor, 2012; Odeyemi, 2014; Helmenstine, 2018). Apart from the hydrocarbons, certain elements such as nitrogen, oxygen, sulfur and metals such as iron, nickel, copper and vanadium could also be found in crude oil.

Crude oil is a non-renewable resource providing energy that has been driving the economy of Nigeria since about six decades when commercial exploitation started. Apart from crude oil being the mainstay of the nation's economy, industries also depend extensively on petroleum derivatives without which they cannot function and produce to optimal capacity. The indiscriminate use and over dependence of man on petroleum and its derivatives have imposed a huge threat on the environment, humans and all forms of lives dependent on the environment through oil spillage, pipeline vandalization and sabotage, oil bunkering and other anthropogenic activities (Jain *et al.*, 2011; Etuk *et al.*, 2013; Odeyemi, 2014, Ikuesan *et al.*, 2019). The environment is said to be polluted with petroleum when crude oil or its derivatives are consciously or unconsciously introduced into the environment at levels harmful to the entire ecosystem (Odeyemi, 2014). Pollution of the environment due to discharge of petroleum and its derivatives into the environment is of greater dimension in the Niger Delta region and therefore a concern to government, environmental researchers as well as the people and residents of oil producing communities resulting in several civil unrests in the region because of the attendant environmental degradation experienced by the communities.

Soil is a key receiver of crude oil spill as well as other different types of hydrocarbons and their derivatives. The sources of crude oil spill differ and amount spilled vary (minor, major or disaster). The effects of crude oil contamination of terrestrial and aquatic environments have been extensively reported by several authors. Ikuesan *et al.* (2019) reported that crude oil pollution no matter the quantity and size caused impaired changes in physicochemical properties, thereby destroying soil integrity and quality. Sharma and Pathak (2017) also asserted that crude oil destroys soil richness, causes alteration in microbiological and physicochemical properties. Agricultural lands have become less productive because crude oil can sterilize the soils and prevent crop growth for varying periods of time (Atlas and Bartha,1992, Onifade *et al.,* 2007; Digha *et al.*, 2017). The overall implication of petroleum hydrocarbon pollution on agricultural land and the socio- economic lives of the people in the affected area include devastation of arable agricultural land resulting in low productive capacity, increased unemployment, poverty and hunger among the people who depend on sales from their farms for food and economy.

The local concentration of spilled petroleum hydrocarbon is affected by the amount spilled, the rate of its release, it's persistence in the environment under various conditions, the extent of its dilution, its mobility, and the rate of biological or non - biological degradation (Odeyemi, 2014). Although, a significant proportion of the hydrocarbons in crude oil is relatively harmless, a number especially the lower molecular weight compounds are toxic or mutagenic (Omotayo *et al.*, 2011).

Consequent upon the effects of crude oil pollution of soil, necessity demands that remediation strategies be put in place in order to ameliorate environmental damages following spill and return polluted soils to their natural state. The overall objective of remediation of polluted ecosystem is the removal or destruction of the contaminants or reduction in toxicity or isolation of the contaminant from the target by interrupting the pathway of exposure. Methods to remove petroleum hydrocarbons have been classified as physical, chemical and biological. Several physicochemical methods have been applied to remediate crude oil polluted soils but with attendant consequences.

Among biological techniques of contaminant removal, bioremediation which is the use of microorganisms to detoxify organic or inorganic xenobiotic compounds from the environment appears to be having upper hand over the physicochemical methods because of its low cost, simplicity, eco- friendliness and ability to halt accumulation of contaminants (Jain *et al.*, 2011; Onuoha *et al.*, 2014). The process relies on the metabolic diversity of some microorganisms to degrade and decrease the concentration of toxic compounds (Onuoha *et al.*, 2014; María *et al.*, 2016). The products of crude oil bioremediation include innocuous substances such as microbial biomass, carbon dioxide and water which are eco-friendly. The process is complex and depends on several factors such as nature and amount of the hydrocarbon present, the prevailing environmental conditions, level of nutrients, aerobic conditions, pH, water and other soil properties and the composition and activity of the indigenous microbial community (Das and Chandran, 2011; Jain *et al.*, 2011 Marinescu *et al.*, 2011; Odeyemi, 2014).

Bioaugmentation, biostimulation and natural attenuation are different bioremediation strategies that can be used in the purification or removal of hydrocarbon pollutant (Lee *et al.*, 2011; María *et al.*, 2016; Buraimoh, 2018). Both organic and inorganic contaminants in soil and underground water can be degraded or immobilized by naturally occurring processes. The toxicity, mass and or mobility of the contaminants can thus be reduced, even in the absence of human intervention. Natural attenuation refers to the decrease in the mass and or concentration of a contaminant without human intervention. Natural attenuation occurs due to sorption, dilution, dispersion, precipitation, ion exchange, volatilization, non-biological transformation, and biological degradation by intrinsic microorganisms. Natural attenuation as of 1995 has been the most common treatment for contaminated groundwater and the second most common treatment for contaminated soil sites. Natural attenuation as a remediation option has been applied to 47 % groundwater sites and 28% of polluted soil sites (Tulis, 1997). The process of natural attenuation takes advantage of a consortium of microbial population as a single microorganism can only degrade limited type of xenobiotics. Also, some hydrocarbons are recalcitrant and can be decomposed only by co- metabolism (Ahmed *et al.*, 2015). The propelling force for petroleum biodegradation is the ability of the microorganisms to utilize the hydrocarbon substrate to satisfy their cell growth requirements and energy needs. The number and composition of soil microorganisms depends on the type of soil, its structure, humidity and the content of organic matter. The indigenous microorganisms must possess the desired metabolic capabilities to degrade the pollutants contaminating the environment. Among natural attenuation approaches, intrinsic bioremediation that is natural bioremediation by indigenous microorganisms is becoming a favored treatment strategy for contaminated sites. This may be because natural processes have been found to be satisfactory for the removal of many pollutants and other more aggressive treatment still do not completely eliminate contaminants or do not result in expected removal or destruction rates (Semprini *et al.,* 1995). Scholars have observed that monitored natural attenuation (MNA) uses the ability of the soil intrinsic microbial community to degrade the contaminant (Alvarez *et al.*, 2011).

When the environment is contaminated with hydrocarbons, bacteria and fungi are the predominant microbial communities that aid the process of hydrocarbon degradation. (Das and Chandran, 2011; Odeyemi, 2014, Unimke, 2018). The ability to degrade and utilize hydrocarbon substrates is exhibited by a wide array of bacterial genera that are widely distributed in oil polluted as well as pristine soils (Malakootian *et al.*, 2009; Abdulsalam and Omale, 2009; Abdulsalam *et al.*, 2011). The efficiency of microorganisms in biodegradation varies and ranged from 6% to 82% for soil fungi, 0.13% to 50% for soil bacteria and 0.003% to 100% for marine bacteria (Das and Chandran, 2011). Many microorganisms found in association with crude oil degradation have been isolated by many researchers. *Achromobacter* spp., *Acinetobacter*, *Arthrobacter*, *Bacillus*, *Sphingomonas, Cellulomonas, Nocardia, Mycobacterium, Corynebacterium, Rhodococcus, Pseudomonas* spp, *Alcaligenes* spp, *Micrococcus* spp were bacteria known with petroleum degradation (Obayori and Salam, 2010). *Fusarium* spp., *Paecilomyces* spp., *Aspergillus, Mucor, Marinobacter* and the yeasts *Candida* spp, *Yarronia* spp., *Pichia* spp have been implicated in hydrocarbon degradation (Odeyemi, 2014). In many environments, there is already an adequate number of indigenous microbial populations that can biodegrade the petroleum pollutant provided that environmental conditions are suitable to favor the degraders (Kim *et al.*, 2005). Therefore, this study aimed at the evaluation of the efficiency of indigenous microorganisms in the remediation of crude oil contaminated soils.

Material and Methods

Sample collection

Soils: Soil samples used in this study were collected from Awoye (5°59' 0"N, 4°55' 0"E), Orioke- Iwamimo (6°11'0"N, 4° 41' 0"E), Oil Palm Plantation at Igodan- Lisa (6° 27' 0" N, 4° 47' 0"), Cassava Plantation Farm at Oba-Ile (7°16'0"N 5°15'0"E) and Cocoa Plantation Farm at Idoani (7° 17' 0"N, 5° 52' 0" E) all in Ondo State, Nigeria. The samples respectively contained Total Petroleum Hydrocarbon (TPH) of 9. 07 mg/kg, 8. 59 mg/kg, 13. 27 mg/kg, 10.57 mg/kg and 11.96 mg/kg (Ikuesan *et al.*, 2016). The samples were collected into sterile black cellophane bags using the hand auger at depth of 15- 20cm. The crude oil used to prepare the experimentally crude oil contaminated soils was standard-grade crude oil (Bonny Light) collected from Bille Flow Station in Port Harcourt, Nigeria.

Preparation of Soil Microcosms and Treatment

The samples were partially air dried at 28 ± 2°C, mechanically homogenized and passed through a 2 mm mesh to remove large particles, debris and stones. Two sets (200 g) of each homogenized and sieved soil samples were used for this study. Triplicate samples of one set were sterilized by autoclaving for three consecutive times at 121°C for 30 min (Bento *et al.*, 2005) and allowed to cool to room temperature. Each set (sterilized and unsterilized) in a sanitized plastic bucket being a microcosm. Sterilized samples were confirmed for sterility using standard methods. Moisture content of each triplicate sample (sterilized and unsterilized) was adjusted to 25% (w/w) with sterilized water and then contaminated with sterile crude oil at 5% (w/w) rate using the method of Ekpo and Ebeagwu (2009) and Njoku *et al.* (2009) and maintained at 28°C ± 2°C. Polluted samples were thoroughly mixed together using sanitized spatula and allowed 7 days for acclimatization between soil and crude oil and the amount of crude oil in sample determined as day zero. Triplicate samples of each set was then evaluated for amount of crude oil degraded at intervals of 30 days for six (6) months using the method described in the section below.

Determination of amount of crude oil in samples.

The weight loss method described by Ijah *et al.*, (2008) and Njoku *et al.* (2009) was used to determine the amount of crude oil in samples. The amount of crude oil in soil samples was determined using air-dried sample to which crude oil had been added. The crude oil remaining in sample was extracted using n- hexane as extractant. Twenty grams (20 g) of each soil sample was mixed with 40 ml of the extractant in a 250 ml Erlenmeyer flask. The flask was then shaken vigorously with mechanical shaker for 30 min to extract the oil (Njoku *et al.*, 2009). The soil-crude oil- n- hexane mixture was allowed to stand for 10 min and then slowly filtered into a pre-weighed beaker through a Whattmann No.1 filter paper. Anhydrous sodium sulphate was spread over the filter paper to remove any moisture present in the mixture. The filtrate was allowed to evaporate by gentle heating at 40°C to a constant weight (Merkl, *et al.*, 2005). The amount of crude oil loss from the soil was then determined as the amount of crude oil added to the soil minus that recovered in the soil at the time of analysis (Njoku *et al.*, 2009). This was then expressed as percentage of crude oil initially present in sample.

Remediation of crude oil by intrinsic microorganisms

This was done by comparing crude oil loss in sterile and non-sterile 5% (w/w) crude oil contaminated soils above. The amount of crude oil biodegraded by intrinsic microbial population (bioattenuation) was the difference in the amount of crude oil recovered from non-sterile and sterile samples on analysis.

Statistical analysis

Data in this study were analyzed by one-way Analysis of Variance (ANOVA) using SPSS version 18.0 (2010). The means were compared by Duncan's Multiple Range Test (DMRT) at 95% confidence level values. Differences were considered significant at $P \leq 0.05$.

Results

Remediation of crude oil contaminated soils by natural attenuation and intrinsic microorganisms.

Results showed that there were reductions in the amounts of crude oil initially present in all the samples. Crude oil loss by natural attenuation was in the order Awoye.

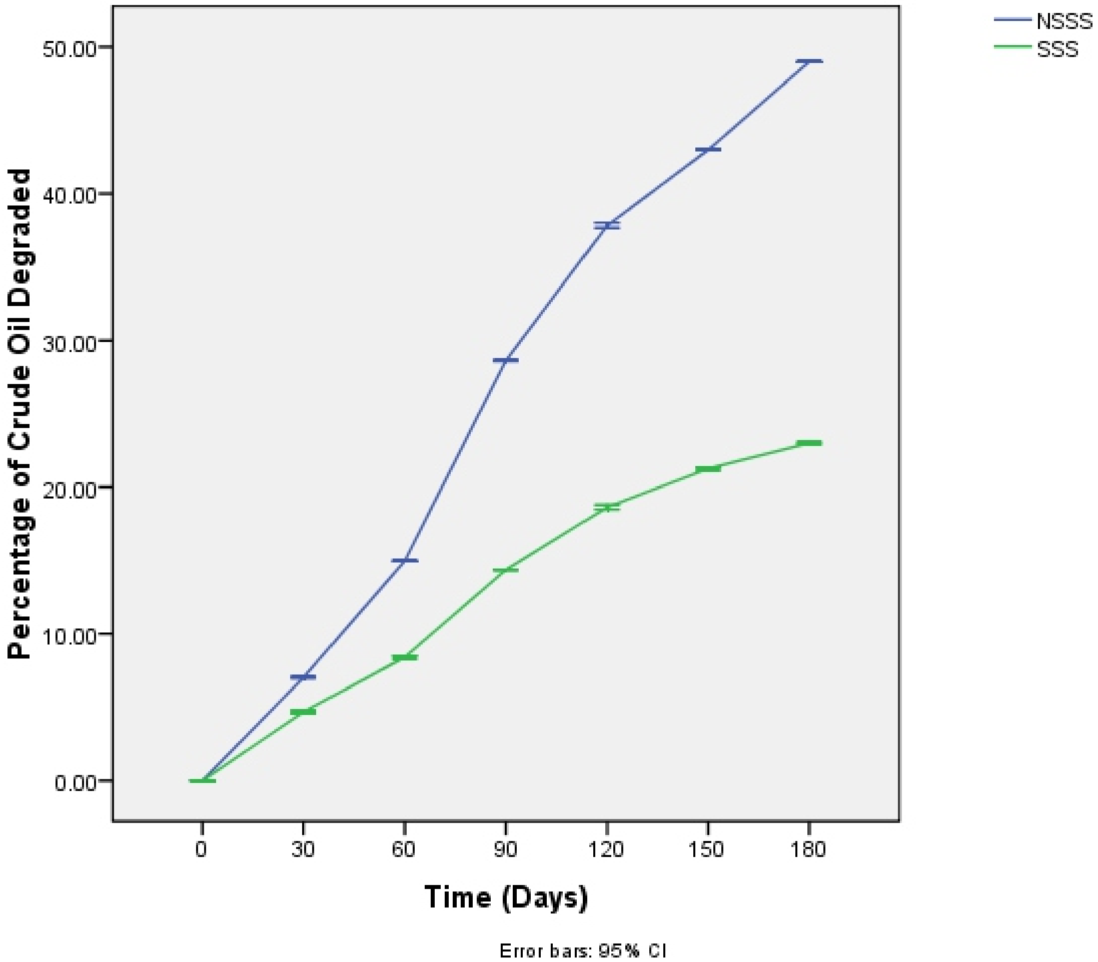

Figure 1a: Remediation of crude oil contaminated Awoye soil by natural attenuation.
Legend: NSS = Non- Sterile Soil Sample, SSS = Sterile Soil Sample

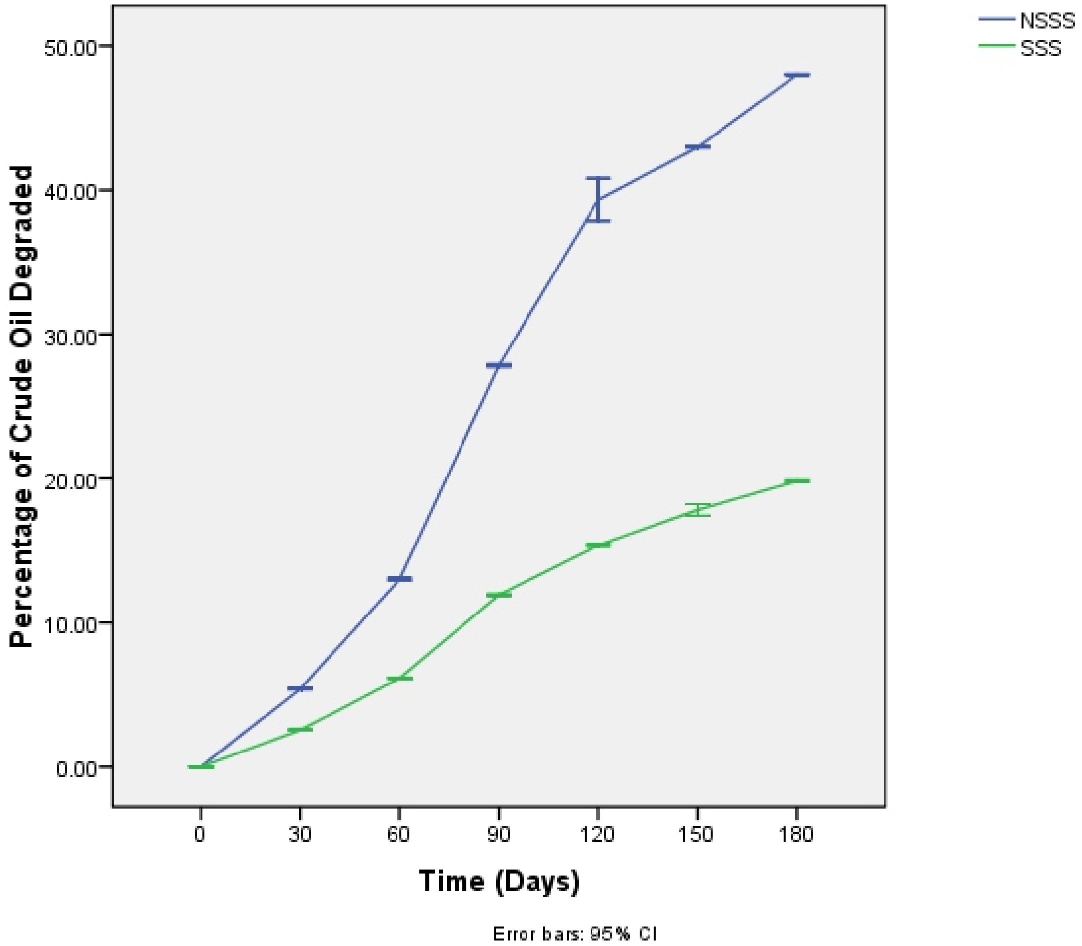

Figure 1b: Remediation of crude oil contaminated Orioke - Iwamimo soil by natural attenuation.
Legend: NSS = Non- Sterile Soil Sample, SSS = Sterile Soil Sample

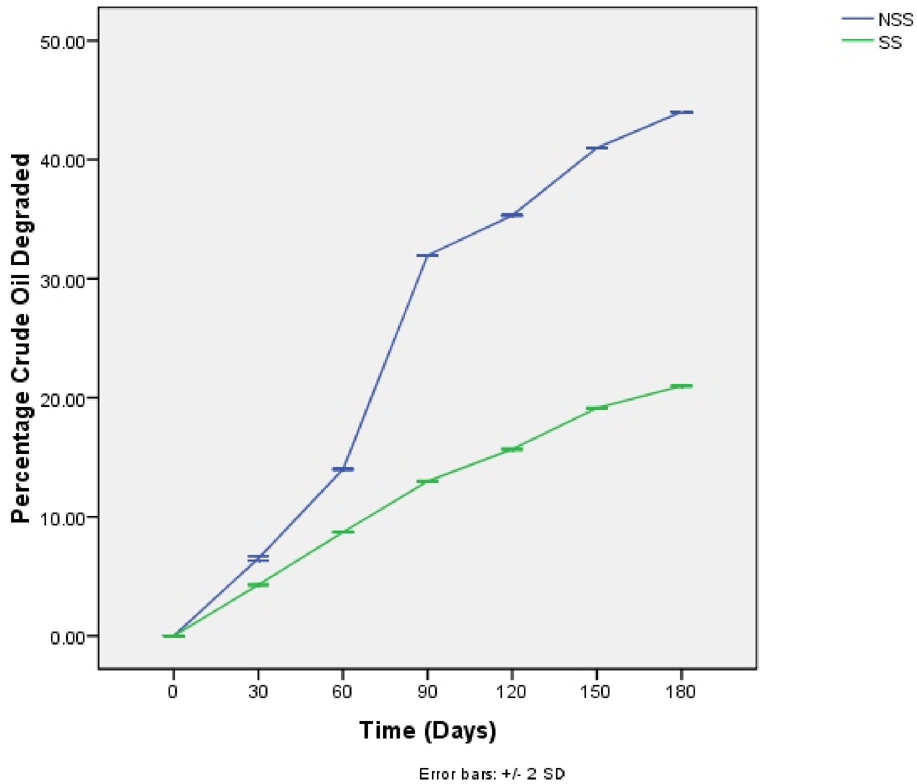

Figure 1c: Remediation of crude oil contaminated Igodan -Lisa soil by natural attenuation.
Legend: NSS = Non – Sterile Soil Sample, SSS = Sterile Soil Sample

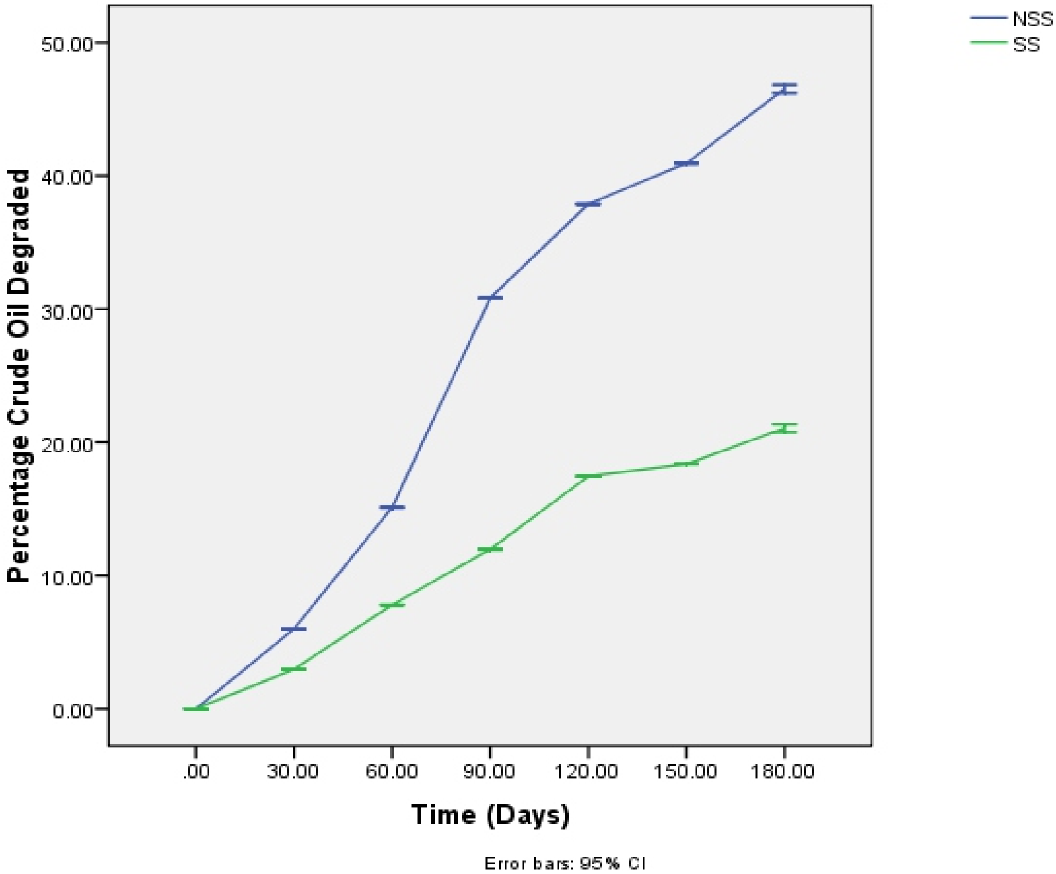

Figure 1d: Remediation of crude oil contaminated Oba - Ile soil by natural attenuation
Legend: NSS = Non – Sterile Soil Sample, SSS = Sterile Soil Sample

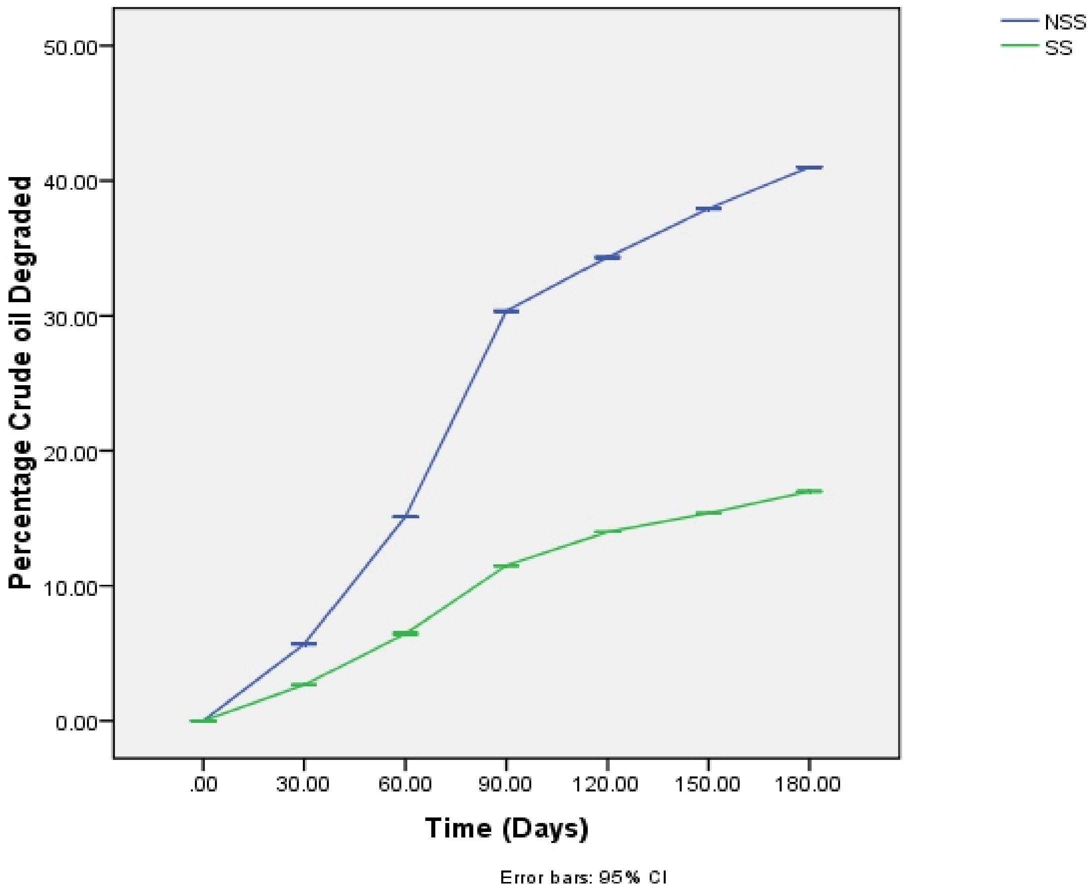

Figure 1e: Remediation of crude oil contaminated Idoani soil by natural attenuation.
Legend: NSS = Non- Sterile Soil Sample, SSS = Sterile Soil Sample

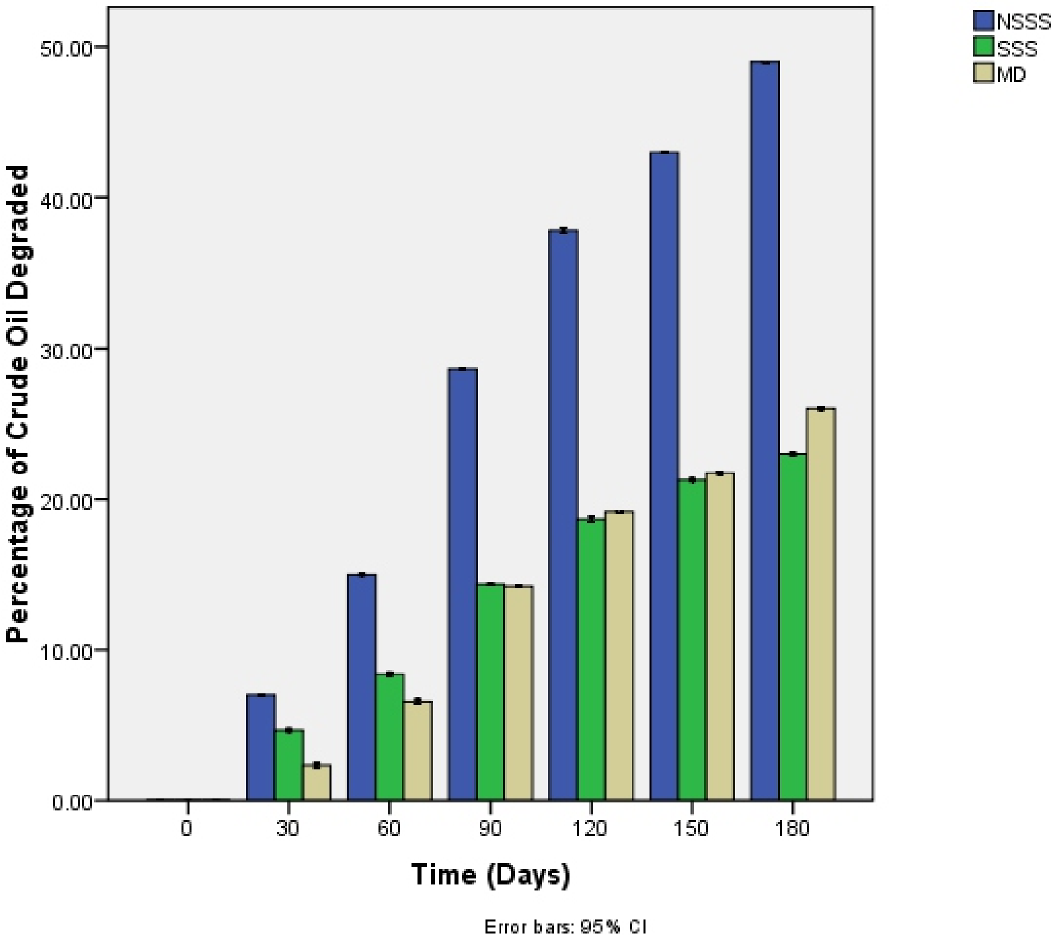

Figure 2a: Remediation of crude oil contaminated Awoye soil by intrinsic microorganisms
Legend: NSS = Non- Sterile Soil Sample, SSS = Sterile Soil Sample, MD = Microbial Degradation

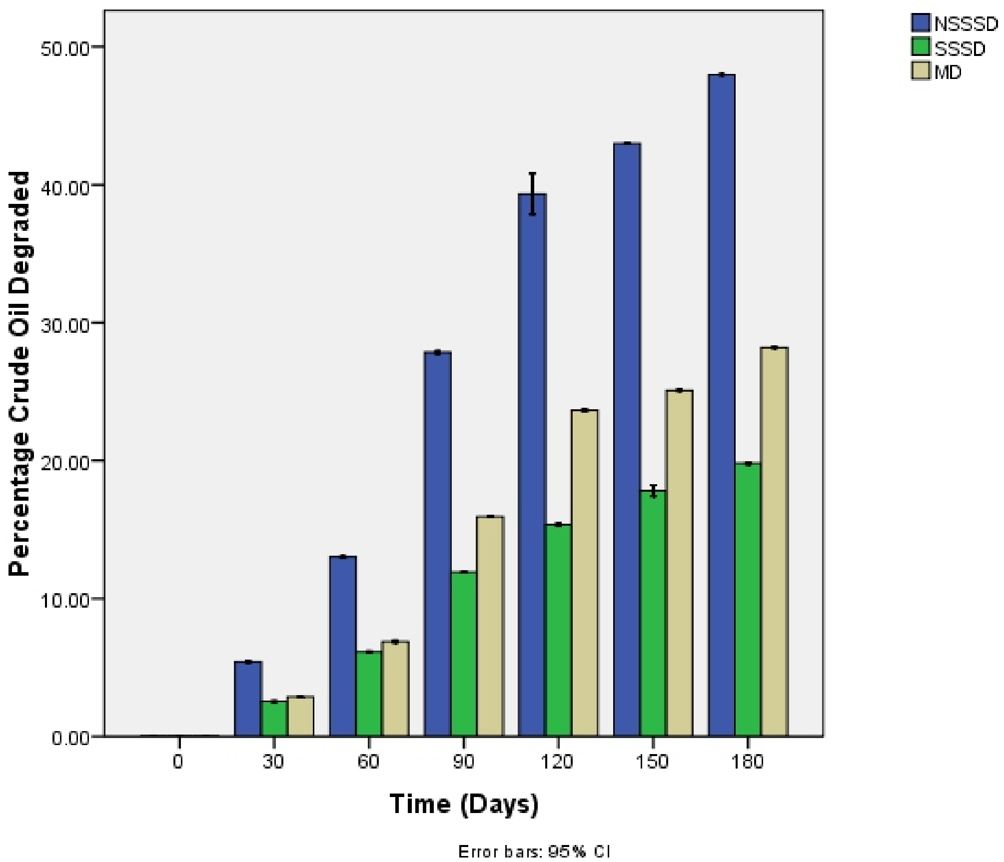

Figure 2b: Remediation of crude oil contaminated Orioke- Iwamimo soil by intrinsic microorganisms
Legend: NSS = Non- Sterile Soil Sample, SSS = Sterile Soil Sample, MD = Microbial Degradation

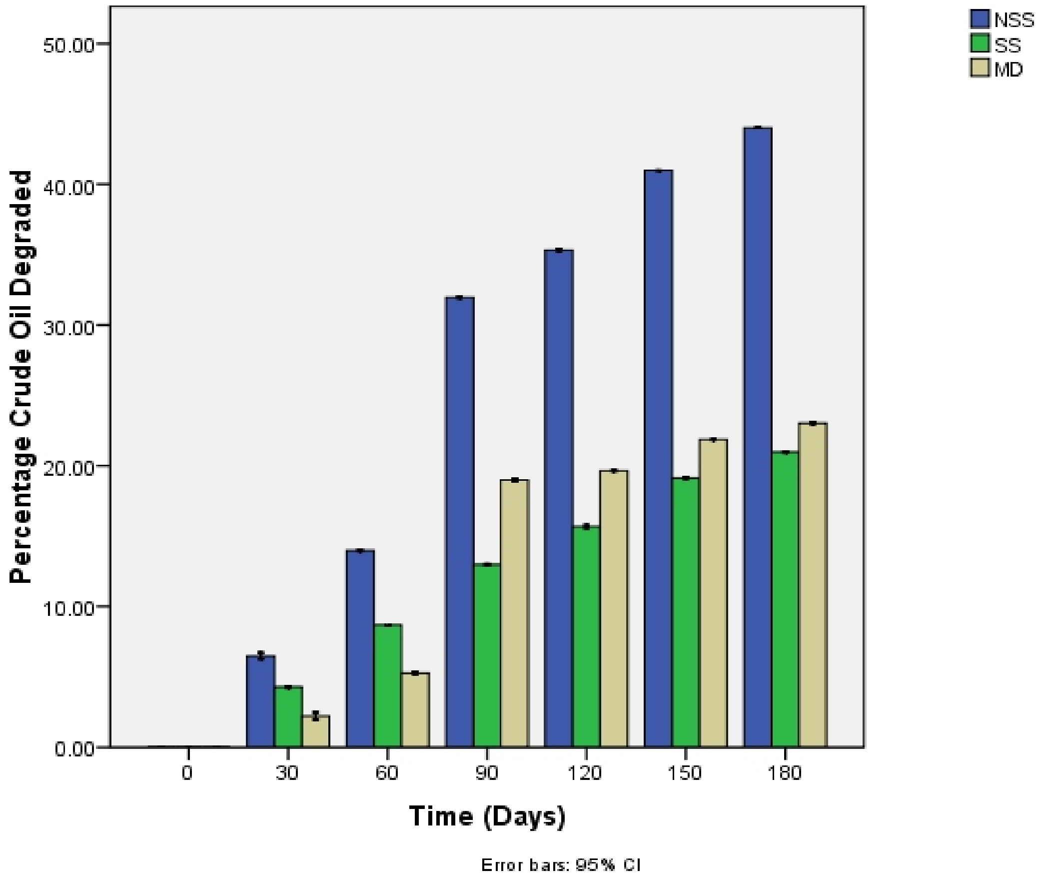

Figure 2c: Remediation of crude oil contaminated Igodan-Lisa soil by intrinsic microorganisms
Legend: NSS = Non- Sterile Soil Sample, SSS = Sterile Soil Sample, MD = Microbial Degradation

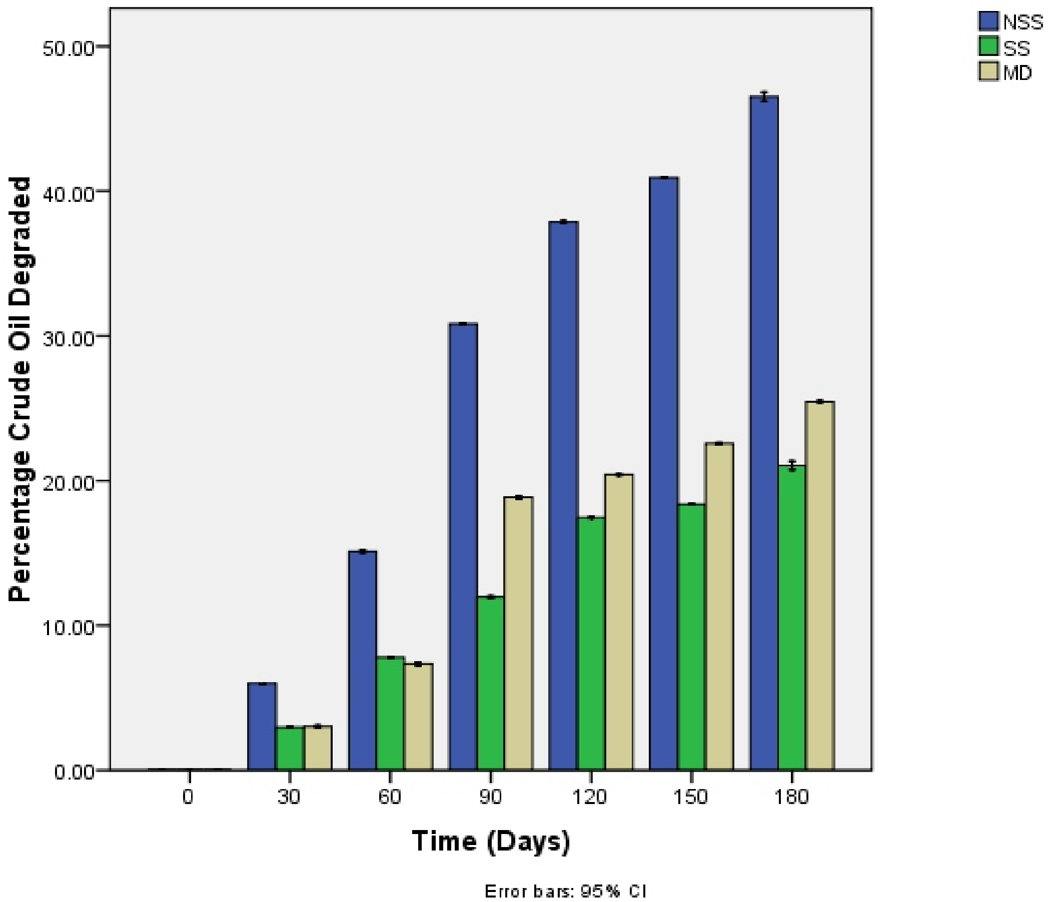

Figure 2d: Remediation of crude oil contaminated Oba - Ile soil by intrinsic microorganisms
Legend: NSS = Non- Sterile Soil Sample, SSS = Sterile Soil Sample, MD = Microbial Degradation

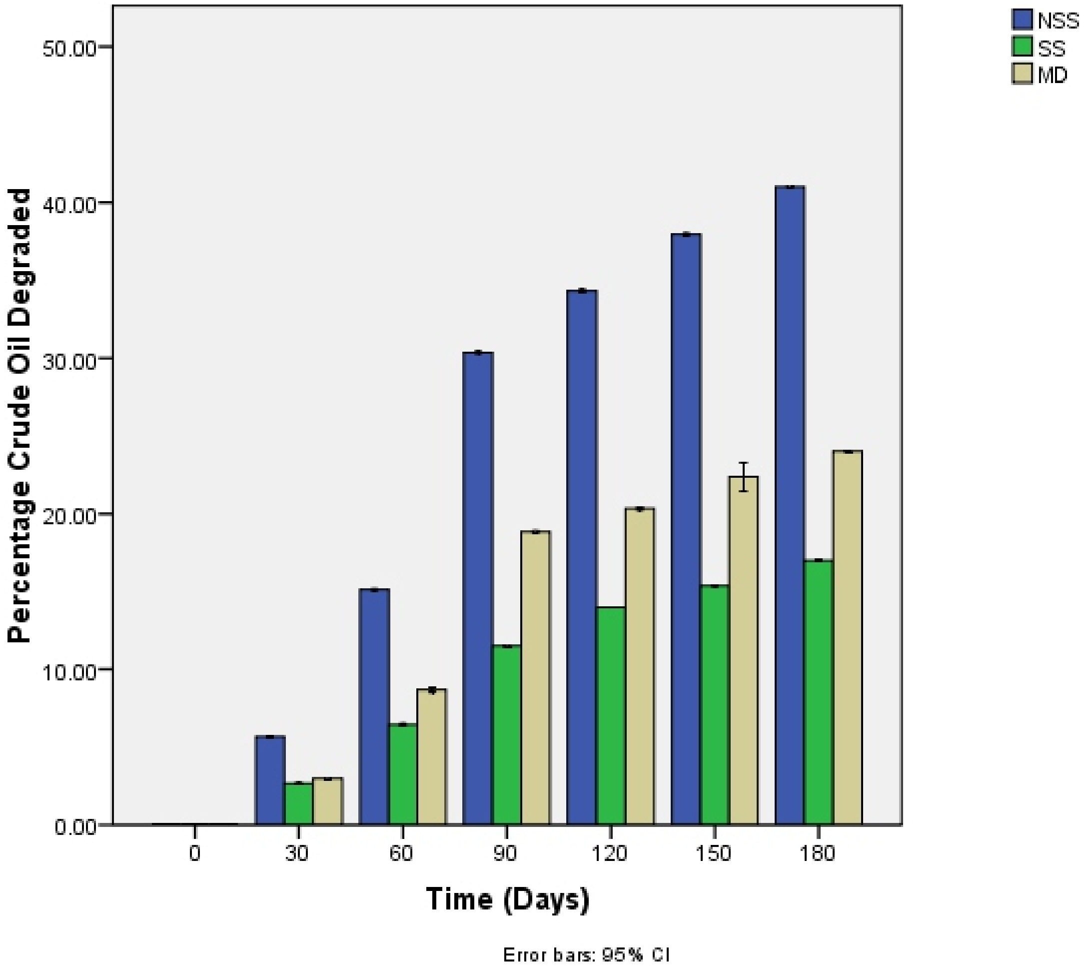

Figure 2e: Remediation of crude oil contaminated Idoani soil by intrinsic microorganisms
Legend: NSS = Non- Sterile Soil Sample, SSS = Sterile Soil Sample, MD = Microbial Degradation

Discussion

Petroleum – based products are the principal source of energy for industry and daily life, providing domestic gases, aviation and fuels and raw materials or feedstock for petrochemical industries (Odeyemi, 2014). The over dependence of man on crude oil and it derivatives have imposed negative impacts on every compartment of the environment, humans and all forms of life.

Crude oil loss was observed in all the treated samples (sterilized and unsterilized). Results revealed that the amount of crude oil loss was higher (41.01 - 49.00%) in non- sterile soils (NSS) than (17.00-22.99%) in the sterile (SSS). The higher amount of crude oil loss in the unsterilized samples could be attributed to the presence of indigenous microorganisms with ability to use crude oil for growth and multiplication. The higher values of crude oil loss in non- sterile samples (41.01-49.00%) compared with the sterile samples is indicative that the soil samples contained indigenous crude oil degrading microbes that used up the petroleum hydrocarbons for cell metabolism and growth. These results agree with the report of Ikuesan (2016) which indicated that the samples contained microorganisms with potentials for crude oil degradation. Results also indicated that degradation of crude oil by intrinsic microbial population constituted 52.33 - 58.74% of degradation by natural attenuation. This implies that microbial degradation by autochthonous microbial community is the principal agent involved in the removal of petroleum hydrocarbon pollutant in the soil. This corroborates the report of Farag and Soliman (2011) that degradation of hydrocarbon by natural population of microorganisms in polluted areas is the main process acting in the depuration of hydrocarbon polluted environment. This study revealed that natural attenuation is a slow process as only 41.01-49.00% of the total concentration of crude oil was removed in six months. The results obtained agree with reports of Odeyemi (2014) and Onuoha *et al.* (2014) that the effects and time required for the reclamation of polluted soil depend on the quantity and concentration of the pollutant. However, results deviated from the report of María *et al.* (2016) which asserted that time was not a limiting factor. This result implies that natural attenuation/bioattenuation can only apply to sites with low concentration of crude oil where no other remedial technique is intended or applicable. The slow removal or degradation process may be due to the presence of certain recalcitrant components of crude oil which are resistant to attack by soil microorganisms. This corroborates the U.S. EPA (1999) guidelines that natural attenuation should not necessarily be considered a stand–alone technology and therefore contingency option(s) must be in place in the event natural attenuation is ineffective or time demanding

Conclusion

The quality of life in an environment depends largely on the overall quality of the environment. This study concludes that crude oil removal efficiency by indigenous soil microorganism under natural condition was more that 50% of the total crude oil loss when soil was polluted with low concentration of petroleum hydrocarbon. However, notwithstanding the time required, hydrocarbon polluted soils can return to its pristine state without human intervention. This implies that indigenous soil microorganisms with ability to degrade crude oil can be isolated and harvested in large biomass, modified and applied as exogenous microbial population and inoculated directly or transferred through supporting materials containing them or by stimulating the indigenous microflora (biostimulation) to enhance the degradation of crude oil polluted agricultural soils in order to speed up crude oil removal efficiency and thus return polluted soil to its pre-contamination status without the application of physicochemical strategies. This becomes expedient because natural removal processes appear too slow.

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References

Abdulsalam, S and Omale, A. B. (2009). Comparisms of biostimulation and bioaugmentation techniques for the remediation of used motor oil contaminated Soil. *Brazilian Arch. Biol. Tech.*, 52: 747-754.

Abdulsalam, S., Bugaje, I. M., Adefila, S. S and Ibrahim, S. (2011) Comparism of biostimulation and bioaugmentation for remediation of soil contaminated with spent motor oil. *Inter. J. Environ. Science Technol.*, 8**:** 187-194.

Agarry, S. E and Ogunleye, O. (2012). Factorial designs application to study enhanced bioremediation of soil artificially contaminated with weathered Bonny light crude oil through biostimulation and bioaugmentation stategy. *J. Environ. Prot.,* 3:748-759

Ahmed, A. B., Nadia, A. A. and Kuther, H. M. (2015). *Ex- situ* bioremediation of soil contaminated with crude oil by use of actinomycetes consortia for process bioaugmentation. *Europ. J. Exp. Biol.*, 5(5): 24-30.

Alvarez, V.M., Marques, J. M. Korenblum, E. and Seldin, L. (2011). Comparative Bioremediation of crude oil- amended tropical soil microcosms by natural attenuation, bioaugmentation or bioenrichment. *Appl. Environ. Sci.* 1-10.

Atlas, R. M. and Bartha, R. (1992). Hydrocarbon biodegradation and oil spill bioremediation. *Adv. Microbiol. Ecol.*, 12: 281-338.

Atlas, R and Bragg, J. (2009) "Bioremediation of marine oil spills: When and when not – the Exxon Valdez experience *Microbial Biotechnol.,* 2(2): 213-221.

Bento, F. M., Camarago, F. A. O. Okeke, B. C. and Frankenberger, W. T. (2005). Comparative bioremediation of soil contaminated with diesel oil by natural attenuation, biostimulation and bioaugmentation. *Biores. Technol.*, 96: 1049-1055.

Buraimoh, O. M., Ogunyemi, A. K., Ibrahim, N. H., Adebusoye, A. S., Ilori, M.O. and Amund O.O. (2017). Efficacy of intervention strategies for bioremediation of crude oil in polluted soil microcosm. *Ife J. Sci.*, 19(2): 303-313.

Cho V., Na J. G., Nlo, N. S., Kim S., and Kim S (2012). Application of saturated, aromatics resins and asphaltenes crude crude oil fractionation for detailed chemical characterization of heavy crude oils by fourier transform ion cyclotron resonance mass spectrometry equipped with atmospheric pressure photoionization. *Energy Fuels.* 26(5): 2558-2565.

Das, N and Chandran, P. (2011). Microbial degradation of petroleum hydrocarbon contaminants. An Overview; *Biotechnol. Res. Inter.*, 11**:** 1 – 13; http://dx.doi.org/10.4061/2011/941810

Digha, O. N., Ambah, B. and Jacob, E. N. (2017) The effects of crude oil spillage on farmland in Gokana Local Government Area of River State. Europ. J. Basic. Appl. Sci. 4(1): 76-96

Ekpo, M. A. and Ebeagwu, C . J (2009). The effect of crude oil on microorganisms and dry matter of fluted pumpkin (*Telfa iria occidentalis*). *Sci. Res. Essay,* 4(8): 733-739.

Etuk, E. A., Ogboi, V and. Nwadinigwe, C. A. (2013). Bioremediation of hydrocarbon polluted soil in the Lowland forest ecosystem in the Niger Delta through enhanced natural attenuation process (ENAP). *Inter. J. Appl. Sci. Technol.,* **3**(8): 128-137.

Farag, S. and Soliman, N. A. (2011). Biodegradation of crude oil petroleum oil and environmental pollutants by *Candida tropicals strain, Brazilian Archiv. Biol. Technol.*, **54** (4): 842-830.

Helmenstine, A. M. (2018). Chemical composition of petroleum. http://www.thoughtco.com/chemical-composition –of- petroleum-607575z1dc

Ijah, U. J. J., Safiyanu, H. and Abioye, O. P. (2008). Comparative study of biodegradation of crude oil in soil Amended with chicken droppings and NPK fertilizer. *Sci. World J.,* 3(2): 63-67.

Ikuesan, F. A, Boboye, B. E. and Adetuyi, F. C. (2016). The microbiological and physicochemical properties of some crude oil contaminated and uncontaminated agricultural soils in Ondo State, Nigeria. *Pyr. J. Microbiol. Biotechnol. Res.*, 2(1): 1-8.

Ikuesan, F. A. (2017). Evaluation of crude oil degradation potentials of some indigenous soil microorganisms. *J. Sci. Res. Rep.,* 13(5): 1-9.

Ikuesan, F. A., Boboye, B. E. and Adetuyi, F. C. (2019). Effects of varying concentration of crude oil on some physicochemical properties of agricultural soil. *J. Sci. Res. Rep.*, 23(5): 1-13.

Jain, P.K., Gupta, V.K., Gaur, R.K., Lowry, M., Jaroli, D.P. and Chauhan, U.K. (2011). Bioremediation of petroleum oil contaminated soil and water. *Res. J. Environ. Toxicol.,* 5(1): 1-26.

Kim, S.J., Choi, D.H., Sim, D.S. and Oh, Y.S. (2005). Evaluation of bioremediation effectiveness on crude oil- contaminated sand. *Chemosphere,* 59: 845-852.

Lee, E-H., Yeon-Sil, K and Cho, K-S. (2011). Bioremediation of diesel-contaminated soils by natural attenuation, biostimulation and bioaugmentation employing *Rodococcus* sp EH831. *Korean J. Microbiol. Biotechnol.*, 39(1): 86-92.

Malakootian, M., Nouri, J. and Houssaui, H. (2009). Removal of heavy metals from paint industry wastewater using leca as an available adsorbent. *Inter. J. Environ. Sci. Technol.,* 6:183- 190.

María, S.V., Oscar, J.H., Juan, A.C., Juan, C.C. and Janet, J. (2016). Approaches for removal of PAH in soils. Soil contamination- Current consequences and further solutions http://dx.doi.org/10.5772/64682

Marinescu, M., Dumitru, M., Lăcătusu, A. and Marinescu, M. (2011). Evolution of maize biomass in a crude oil polluted soil according to the treatment. Applied scientific papers, UASVM. Bucharest, LIV**:** 287-292.

Merkl, N. Schutze- Kraft, R and Infante, C. (2005). Phytoremediation in the tropics- influence of heavy crude oil on root morphology characteristics of graminoids. In Phytoremediation of petroleum- contaminated soils. Merckl, N. (Ed), Margraf Publisher, Weikershim, pp: 53-59.

Njoku, K. L., Akinola, M. O. and Oboh, B. O. (2009) Phytoremediation of crude oil contaminated soil: The effect of growth of *Glycine max* on the physico-chemistry and crude oil contents of soil. *Nat. Sci.*, 7(12): 79 - 87.

Obayori, S. O., Salam, L. B.(2010) Degradation of polycyclic aromatic hydrocarbons: Roles of plamids. *Sci. Res. Ess.* 5:4093 - 4106

Odeyemi, O. (2014). Two centuries of oil and gas (1860- 2060). Available: www. universalacademicservices.org,

Omotayo, A.E., Efetie., O.A., Oyetibo, G., Ilori, M.O. and. Amund, O.O. (2011). Degradation of aviation fuel by microorganisms from tropical polluted soils. *International J. Biol. Chem. Sci.*, 5(2): 698-708.

Onifade, A. K.,. Abubakar, F. A and Ekundayo, F. O. (2007). Bioremediation of crude oil polluted soil in the Niger Delta Area of Nigeria using enhanced natural attenuation. *Res. J. Appl. Sci.,* 2(4): 498-504.

Onuoha, S. C., Chukwura, E. I. and Fatokun, K. (2014). Stimulated biodegradation of spent lubricating motor oil in soil amended with animal droppings. *Ame. J. Biosci.*, 2(1): 19-27.

Sharma, S. and Pathak, H. (2017). Physicochemical properties of petroleum contaminated soil collected from arid region of Rajasthan (Churu). *Inter. J. Pharma. Bio. Sci.*, 8(2): 926-932.

Semprini, L., Kitanidis, P., Kampbell, D. and Wilson, J. (1995). Anaerobic transformation of chlorinated aliphatic hydrocarbons in a sand acquifer based on spatial chemical distributions. *Wat. Res.*, 31: 1051-1062.

Sinnathambi, C. M. and Nor, M. N. (2012). Relationship between SARA fractions and crude oil fouling. *J. Appl. Sci.*, 12: 2479-2483.

Tulis, D. S. (1997). Issues associated with natural attenuation. U. S. Environmental Protection

Agency, Washington, D.C.

Unimke, A.A., Mmuoegbulam, O.A. Anika, O.C. (2018). Microbial degradation of petroleum hydrocarbons: Realities, challenges and prospects. *Biotechnol. J. Inter.*, 22(2): 1-10.

U. S. Environmental Protection Agency (1999) Information on OSWER directive 9200. Use of monitored natural attenuation at superfund, RCRA Corrective action, and underground storage tank Sites U. S. EPA.