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EVALUATION OF THE EFFECT OF CRUDE OIL AND KEROSENE ON SOIL MICROBIAL POPULATION

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ABSTRACT

The study was undertaken to investigate the effect of soil contamination with crude oil and kerosene on microbial population and biodiversity. The effects of crude oil and kerosene on soil microbial population were investigated by contaminating soils at five loading rates (1.0, 5.0, 10, 15, 20 %) volume of oil/weight of soil and monitoring activity at 7 days interval. The highest level of average fungal and bacterial count in crude oil contaminated soil was at 21 days, the average count of the fungal count was 126 × 10^4cfu /g of soil, while that of bacterial was 143 × 10^6cfu/g of soil. The highest level of average fungal and bacterial count in kerosene contaminated soil was at 14 days, the average fungal count was 102 × 10^4cfu /g of soil while that of bacterial count was136 × 10^6cfu/g of soil. Analysis of variance of the average count of fungi and bacteria showed a high significant difference between the control and the oil treated soils at $p < 0.05$ level. Species of twelve fungal and eight bacterial genera were isolated from the soils. The order of fungal and bacterial is a reverse of the decreasing order of fungal diversity of these same soils. This showed that higher concentrations of crude oil have an adverse effect on fungal diversity while enhancing the population of fewer fungi.

KEYWORDS: *Crude Oil, Kerosene, Bacteria, Fungi, Pollution*

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INTRODUCTION

The World Health Organization (WHO) has estimated that 4.9 million deaths (8.3 per cent of total mortality worldwide) are attributable to environmental exposure and inappropriate serious management of toxic chemicals (Pruss-Ustun*et al.,* 2011). Environmental pollution has been on the rise in the past few decades owing to increased human activities on energy reservoirs, unsafe agricultural practices and rapid industrialization (Hadia and Ahmed, 2018). Amongst the pollutants that are of environmental and public health concerns due to their toxicities are: heavy metals, nuclear wastes, pesticides, greenhouse gases, and hydrocarbons.

Environmental pollution associated with petroleum hydrocarbons is one of the world's most common environmental problems (Xu *et al.,* 2018; Benal *et al.,* 2014), petroleum oil spillage is one of the most serious environmental problems currently facing the oilproducing areas and occurs in large scale in some communities. The oil spillage could be attributed to different causes such as accidental spills, leakage, and vandalization of pipelines and corrosion of pipelines which allow the seepage of crude oil into the environment (Wang *et al.,* 2018). The effect of oil spillage on land has become a global issue as land play an important role in the sustenance of man (Abii and Nwosu, 2009). When land is contaminated, the contaminants

change the chemical and biological properties of the soil, toxic to some soil microorganisms. (Udeani*et al.,* 2009; Hentati*et al.,* 2013;Xu *et al.,* 2018). The chemical composition of crude oil and kerosene varies significantly and can have diverse effects on different organisms within the ecosystem and these differences are due to variation in concentration levels of the various constituents (Srerdrup *et al*., 2003).

The contamination changes the physiochemical and biological properties of the soil because the oil may be toxic to some soil microorganisms and plants (Minai-Tehrani and Herfatmanesh, 2007). Environmental pollution with petroleum and petroleum products (a complex mixture of hydrocarbons) has been recognized as one of the most serious current problems especially as when associated with accidental spills on a large scale. Contamination of soil by crude oil could lead to reduced microbial density and activities.

Soil conditions of agricultural land, microorganisms as well as plants are damaged or altered by any contact with crude oil (Onuoha*et al.,* 2003). Excess oil in soil limits the availability of nitrogen (John *et al*., 2010). Soils that are polluted with petroleum hydrocarbons (PHCs) are different from unpolluted soils due to changes in their biological as well as physicochemical properties (Robertson *et al*., 2007; Nwaoguikpe, 2011; Akpoveta*et al*., 2011).

Petroleum hydrocarbon may interfere with the plant-fungus relationship by altering the soil environment so that movement of diffusible chemical signals such as auxins is prevented. It may also affect this relationship by altering the root exudation pattern (Kirk *et al*., 2005).

Soil biological activity, including soil microbial biomass, is influenced by a range of physiochemical, environmental parameters and perturbations. Therefore, soil microbial activity may be used to assess disturbed soil (Labud *et al.,* 2007).

Biologically and biochemically mediated processes in soils are of utmost importance to ecosystem functions (Tejada *et al.,* 2011; Lopes *et al.,* 2011). There is a huge diversity of organisms belonging to different taxonomic and physiologic groups that interact at different levels within the community in soil biota (Dombrowski *et al.,* 2016; Dvorak *et al.,* 2017; Lopes *et al.,* 2011). In this biota, soil microorganisms constitute a source and are the driving force behind many soil processes, including the transformation of organic matter, nutrient release, transformation of C, N, P and S, degradation of xenobiotic compounds, the formation of soil physical structure and enhanced nutrient uptake by plants (Chen *et al.,* 2010; Lopes *et al.,* 2011).

Bioremediation processes utilize naturally occurring microorganisms to treat specific environment polluted with chemicals (Suja *et al.,* 2014). Bioremediation process using fungi and bacteria can lead to complete degradation of the petroleum hydrocarbon contaminants in the soil environment (Bento *et al.,* 2005; Achal *et al.,* 2011; Yang et al., 2015). Various soil microorganisms have great potential for bioremediation (Guerra *et al.,* 2018). They degrade organic pollutants by using them as their carbon and energy source. And more than 200 species of bacteria, fungi, and even algae are capable of degrading hydrocarbons because of their ubiquitous nature. Various genera of microorganism that contain hydrocarbon degrading species; *Pseudomonas*, *Vibrio*, *Corynebacterium*, *Arthrobacter*, *Brevibacterium*, *Staphylococcus*, *Bacillus*, *Thiobacillus*, *Penicillium*, *Candida*, *Fusarium*, *Aspergillus*, *Talaromyces* and *Articulosporium* (Snape *et al*. 2001). Onwurah (2003) reported that *Pseudomonas, Micrococcus* and *Bacillus* can metabolized the toxic components of crude oil, leading to degradation. Nakamura *et al.,* (2007) and Hozumi*et al.* (2000) reported the isolation of organisms with high potential for degrading oil with high viscosity after an oil spill. Some Fungi and bacteria may appear resistant to PHC (Nicolotti and Egli,199; Lea-Smith *et al.,* 2015).

Most bacteria isolated in large numbers from many oil polluted waters and soils are aerobic bacteria such as *Pseudomonas*, *Mycobacterium*, *Rodococcus*, *Arthobacter*, *Acinetobacter*, *Nocardia*and *Bacillus* (Okoh, 2003; Zhang *et al.* 2010). Some fungi also have the ability to degrade organic pollutants. For instance, white rot fungus (*Planerochaetechrysosporium*) is an example of ligninolytic fungi capable of degrading polyaromatic hydrocarbons and other harmful environmental pollutants (Pal *et al*., 2010) *Cunninghamellaechinulata*andmycorrhizal fungi have also been used for the remediation of PHC-polluted soil (Alarcon *et al*., 2008). *Aspergillus*sp.; *Cephalosporium*sp.; *Cladosporium*sp.; *Fusarium*sp.; *Geotrichum*sp.; *Mucor*sp.; *Penicillium*sp.; *Curvulariasp* and *Trichoderma*sp. and yeast isolates - *Candida* sp. and *Rhodotolura*sp (Obire and Anyanwu, 2009). Okerentugba and Ezeronye (2003) demonstrated the ability of *Penicillium* spp., *Aspergillus*spp. and *Rhizopus*spp. to degrade petroleum hydrocarbons. Chaudhry*et al.* (2012) further reported that the advantages associated with fungal bioremediation lay primarily in the versatility of fungi in utilizing petroleum hydrocarbon. Adekunle and Adebambo (2007) demonstrated the ability of *Aspergillusniger*, *A. flavus*, *Mucor*spp., *Rhizopus*spp. and *Talaromyces*spp. to utilize and degrade crude oil and other petroleum products such as diesel, kerosene, spent and unspent engine oil. Similarly, Uzoamaka*et al.* (2009) isolated *Aspergillusversicolor*,*Aspergillusniger*, *Aspergillusflavus*,*Syncephalastrum*spp.,*Trichoderma*spp., *Neurosporasitophila*, *Rhizopusarrhizus*and*Mucor*spp from oil contaminated soil and demonstrated their potentials for hydrocarbon biodegradation. Using fungi can lead to complete degradation of the petroleum hydrocarbon contaminants in the soil environment (Bento *et al.*, 2005, Achal*et al.*, 2011).

For bioremediation to be effective there must be contact between the microorganisms and the pollutants and since various types of pollutants exist in a PHC-polluted soil, a wide range of microorganisms is required for effective bioremediation (Xu*et al.*, 2018). For these reasons, the importance of microorganisms is unquestionable in the maintenance of quality and productivity of agricultural soils. This study was carried out with the objective to evaluate bacterial and fungal populations in the soil contaminated with crude oil and kerosene.

MATERIALS AND METHODS

Bonny light crude oil was collected from Exxon Mobil, Eket in AkwaI bom State and kerosene was collected from Nigeria National Petroleum Corporation (NNPC) station, Uyo in AkwaI bom State, Nigeria. Soil samples were randomly collected with the aid of auger from the University of Nigeria, Nsukka agricultural farmland. The soil samples collected were bulked, air dried and sieved to remove coarse fragments. Soil sample (100 g) was weighed into a conical flask and amended with crude oil and kerosene oil (0%, 1%, 5%, 10%, 15% and 20%, volume per weight), respectively. The oil was thoroughly mixed with the soil in the conical flask. Soil sample amended with crude oil (0%, 1%, 5%, 10%, 15%, and 20 %, v/w) and kerosene oil (0%, 1.0%, 5.0%, 10%, 15%, and 20 %, v/w), in conical flasks were plugged with cotton wool**.** Each set up was arranged in triplicate, incubated at 28^0C, analyzed at 0, 7, 14, 21 and 28 days respectively for the microbial load.

Physico-Chemical Studies of the Soil

Particle size analysis was determined by the Bouyoucos hydrometer method of Gee and Bauder (1987)**.** Fifty grams of soil sample was weighed into a 500 ml conical flask and plugged with cotton wool. Fifty ml of Calgon (a mixture of sodium hexametaphosphate and sodium carbonate) was dispensed into the conical flask containing the soil sample. Two hundred ml of distilled water was added, stirred thoroughly with a glass rod and allowed to stand overnight, followed by agitation for 30 minutes. After agitation, the mixture was transferred to a 1000 ml measuring cylinder using a wash bottle. Hydrometer used was placed gently in the suspension and the volume made up to 1000 ml. The hydrometer was removed and the cylinder inverted 3-4 times with the palm covering the mouth. The cylinder was placed on the bench and the hydrometer

re-immersed. The first hydrometer reading and temperature were taken after 40 seconds. After 2 hrs the second hydrometer reading and temperature were taken. The suspension was decanted and the sediments transferred into a 250 ml beaker using a wash bottle and subsequently dried at 105^0C for 2 hrs. After drying the soil and sieved using 0.25 mm sieve, the coarse sand was weighed.

The particle size (clay, silt, and fine sand) fraction was determined using:

$$Clay\ fraction = \frac{2\ hours\ reading\ of\ hydrometer}{weight\ of\ soil\ sample} \times \frac{100}{1}$$

$$Silt\ fraction = \frac{1st\ hydrometer\ reading - 2nd\ hydrometer\ reading}{Weight\ of\ soil\ sample} \times \frac{100}{1} = \%\ silt$$

Total Sand = 100 - % Clay - %Silt

% Coarse sand = weight of coarse sand multiplied by 2.

Fine sand = % total sand -% coarse sand.

pH was determined according to Black (2000) using a pH meter inserted into a partially settled suspension and stirred occasionally with a glass rod. Soil moisture was determined according to Black (2000). Two porcelain basins were weighed and the weight recorded. Twenty grams of each of the wet soil samples were weighed in duplicates into each basin. The samples were dried in an oven at 105^oC for 24 hours and later cooled in desiccators. The dry sample was re-weighed and the weight obtained by subtracting the weight of the empty basin from the combined weight of the basin and the dry soil. The gravimetric moisture content was obtained using the equation:

$$\theta g = \frac{m-d}{d}$$

Where θ g = moisture content

m = mass of moist soil prior to drying

d = mass of the same soil after drying

The percentage of moisture was obtained using the relation below; .

$$\%\ moisture\ was\ obtained = \frac{weight\ of\ moisture}{weight\ of\ oven\ dry\ soil} \times \frac{100}{1}$$

Total organic carbon and percentage organic matter in soil was determined by the wet oxidation method of Walkey and Black (1934) and the results were calculated according to the following formula:

$$\%\ \text{Organic carbon in soil} = \frac{(\text{MeK}_2\text{Cr}_2\text{O}_7 - \text{MeFeSo}_4) \times 0.003 \times 100 \times \text{F}}{\text{g of air-dry soil}}$$

Correction factor, F = 1.33

Me =Normality of solution x ml of solution used.

% organic matter was determined by multiplying % organic carbon (% C) by 1.724, that is, $\%\ C \times 1.724$.

Soil nitrogen was determined by the modified Kjeldahl method (Bremner, 1965) and the percentage of N in the sample was determined from the equation;

$$Percentage\ N = \frac{T \times N \times 14.01}{1000} \times \frac{100}{Ws}$$

Where T = Sample titre

N = Normality

Ws = Weight of sample

N = Normality of EDTA

Ammonium acetate method (Jackson, 1970) was used to determine the exchangeable bases.

The exchangeable bases (Sodium, Potassium, calcium, and Magnesium) were determined from the equation;

$$Meq = \frac{Mg}{100\ g\ soil} = T \times N \times \frac{vol}{Aliq} \times \frac{100}{Ws}$$

Where Meq= milliequivalents of charge per 100 g of dry soil

T = Sample titre

N = Normality of EDTA

Vol = Volume of leachate collected

Aliq = ml aliquot titrated

Ws = Weight of sample leached

Cation exchange capacity (CEC) was determined using the equation;

$$\text{Meq:}\frac{\text{CEC}}{100\ \text{g}}\text{soil} = \text{T} \times \text{N} \times \frac{\text{Vol}}{\text{Aliq}} \times \frac{100}{\text{Ws}}$$

Na+ and K+ were determined calorimetrically using Flame‑photometer with 1.00N NH4 OAC leachate.

Determination of exchangeable acidity(Mclean, 1965) was determined from the equation below;

Calculation for exchangeable acidity (EA);

$$\text{Meq:}\frac{\text{EA}}{100\ \text{g}}\text{soil} = \text{T} \times \text{N} \times \frac{\text{Vol}}{\text{Aliq}} \times \frac{100}{\text{Ws}}$$

Phosphorus in the soil sample was also determined.

Microbiological Analysis

Media preparation for Nutrient agar (NA) was carried out by dissolving 28g of dehydrated nutrient agar powder in 1liter of distilled water and mixed to dissolve. The medium was then sterilized in the autoclave at 121 ^{o}C and 15 psi, and dispensed into sterile Petri dishes and allowed to gel while the Sabouraud dextrose agar (SDA) was prepared by dissolving 62g of the powder in 1 liter of distilled water, sterilized in an autoclave at 121 ^{o}C and 15 psi and dispensed into sterile Petri dishes and allowed to gel.

The microbial load was determined by serial dilution of soil suspension and the desired dilutions plated on nutrient agar (NA) and sabouraud dextrose agar (SDA). The bacteria and fungi were incubated by pour plate technique, the incubation was done at room temperature for 24 to 48 hours and 4 to 5 days for bacteria and fungi respectively. Counts were recorded from duplicate plates as colony forming units/g.

Pure bacterial isolatesstored at 4^{o}C on agar slants were identified using morphological and biochemical techniques, motility, Gram staining and spore staining using standard bacteriological techniques as described by Cheesbrough (2006),

Amadi (2009) and the taxonomic scheme of Bergey's Manual of Determinative Bacteriology (Holt *et al.*,1994).

A motility test was done to determine the presence or absence of flagella in the bacterial isolates. A small drop of the suspension of the isolate was placed on a slide and covered with a coverslip. The preparation was examined microscopically for motile organisms using the x10 and x40 objective lenses (Cheesbrough, 2006). Gram stain was done to identify and differentiate between Gram positive and Gram-negative bacteria using the method described by Cheesbrough (2006). The spore staining test was carried out to determine and observe the spore-forming bacteria (Onyeagba, 2004).

Physicochemical Properties of Soil

The soil was identified and classed as sandy loam. The pH of the soil was acidic: 4.55 ± 0.49; moisture content: 23.97%; organic carbon: 0.99% ; organic matter: 1.17% ; nitrogen: 0.098%; clay and silt: 32%; fine sand: 36%; coarse sand: 40%; saturated base: 32.82%; Phosphurus: 31.71 ppm; cation exchange capacity: 14.80 (meq/100 g); exchangeable acid: 2.80 (meq/100 g) and exchangeable base meq /100 g; sodium, 0.028; potassium: 0.230; calcium: 2.80 and magnesium: 1.80 (meq /100 g).

Table 1: Physiochemical Properties of Soil Sample

Parameter	Values(0- 15cm Depth)
Texture class	Sandy loamy
Particle size (Clay & Silt)	32 %
Particle size (Fine sand)	36 %
Coarse sand	40 %
pH value	4.55±0.49
Moisture content	23.97 %;
Carbon	0.99 %
Organic matter	1.72 %
Nitrogen	0.098 %
Exchangeable bases:Sodium	0.028 (meq /100 g)
Potassium	0.230 (meq /100 g)
Calcium	2.80 (meq /100 g)
Magnesium	1.80 (meq /100 g)
Cation exchange capacity	14.80 (meq /100 g)
Saturated base	32.82 %
Exchangeable acidity	2.80 (meq/100 g)
Phosporus	31.71 ppm

Fungi Isolated and Bacteria Isolated

Twelve pure fungal isolates with different morphological characteristics were successfully isolated from the soil samples contaminated with crude oil and kerosene. The fungal isolates were successfully grown, identified and characterized morphologically. The fungal isolates showed differences in morphological appearance, pigmentation, and sporulation in different media. Based on the macroscopic and microscopic morphological characteristics, the twelve fungal isolates belong to the genera *Aspergillu, Alternaria, Candida, Curvularia, Fusarium, Penicillium, Mucor,Cephalosporium, Trichoderma, Cladosporium, Rhizopus*spand *Rhodotorula*sp.

Eight pure bacterial isolates with different morphological and biochemical characteristics were successfully isolated from the soil samples contaminated with used crude oil and kerosene. All the bacterial isolates were successfully grown and identified based on their Gram stain reaction, spore stain reaction, motility, and biochemical reaction and with reference to Bergey's Manual of Determinative Bacteriology. The eight bacterial isolates belong to the genera. *Pseudomonas, Bacillus,*

Staphylococcus, Streptococcus, Escherichia sp, *Micrococcus, Klebsiella, Corynebacterium.* Among all the bacterial isolates *Pseudomonas* and *Bacillus* obtained were the most common.

Fungal Count in Soil Contaminated with Crude Oil and Kerosene

The average count of total fungi (colony forming unit /gram) for 0 % crude oil treatment was 3.2×10^5 at 0 day; 3.8×10^5 at 7 days; 4.2×10^5 at 14 days; 4.8×10^5 at 21 days and 4.5×10^5 at 28 days. For 1 %, 3.0×10^5 at 0 day; 5.1×10^5 at 7 days; 6.9×10^5 at 14 days; 8.5×10^5 at 21 days and 5.6×10^5 at 28 days. For 5 %, 2.7×10^5 at 0 day;6.4×10^5 at 7days; 7.5×10^5 at 14 days; 9.7×10^5 at 21 days and 7.0×10^5 at 28 days. 2.4×10^5 at 0 day; 7.2×10^5 at 7 days; 8.1×10^5 at 14 days; 1.02×10^5 at 21 days and 8.2×10^5 at 28 days for 10 %. 2.1×10^5 at 0 day; 7.8×10^5 at 7 days; 9.9×10^5 at 14 days; 1.18×10^6 at 21 days and 9.8×10^5 at 28 days for 15 %. 1.7×10^5 at 0 day; 8.3×10^5 at 7 days; 1.04×10^6 at 14 days; 1.26×10^6 at 21 days and 1.13×10^5 at 28 days for 20 % crude oil treatment respectively. The average count of total fungal (colony forming unit /gram) at 0 % kerosene treatment was 2.8×10^5at 0 day; 3.2×10^5 at 7 days; 4.4×10^5 at 14 days; 4.2×10^5 at 21 days and 3.9×10^5 at 28 days. For 1 %, 2.9×10^5at 0 day; 4.1×10^5 at 7 days; 6.0×10^5 at 14 days; 5.3×10^5 at 21 days and 4.9×10^5 at 28 days. For 5 %, 2.5×10^5 at 0 day; 4.7×10^5 at 7days; 7.2×10^5 at 14 days; 6.5×10^5 at 21 days and 5.8×10^5 at 28 days. 2.2×10^5 at 0 day; 5.0×10^5 at 7 days; 8.8×10^5 at 14 days; 8.2×10^5 at 21 days and 6.4×10^5 at 28 days for 10 %. 2.0×10^5 at 0 day; 5.8×10^5 at 7 days; 9.4×10^5 at 14 days; 8.9×10^5 at 21 days and 6.8×10^5 at 28 days for 15 %. 1.9×10^5 at 0 day; 6.3×10^5 at 7 days; 1.02×10^6 at 14 days; 9.5×10^5at 21 days and 8.7×10^5 at 28 days for 20 % kerosene treatment respectively.

Table 2: Bacterial and Fungal Isolates

Bacterial	Fungal
Pseudomonas spp. Bacillus spp.	Aspergillusspp. Penicillium spp.
Staphylococcus spp. Streptococcus spp. Escherichia coli Micrococcus spp. Klebsiellaspp. Corynebacteriumspp	Mucor spp. Alternariaspp. Trichoderma spp. Candida spp. Curvulariaspp. Fusariumspp. Rhizopusspp. Rhodotorulaspp Cladosporiumspp Cepholosporiumspp

Table 3: Morphological and Microscopic Characterization of Fungal Isolates

Organism	Morphological Characteristic	Microscopic Examination
Aspergillusspp	Yellow-green,blue-green,grey-green,red-brown, yellow,white filamentous growth that turn black sporulation	Long septate hyphae with swollen conidiophore bearing phialide at its apex
Penicillium spp	Green, white, powdery yellow, with raised rough surface colonies	Septate and branch conidiophore with brush like conidial head
Mucor spp	White wooly growth that turns darker as it sporulates	Non septate hyphae with straight sporangiophoressherical spores
Alternariaspp	Olivaceous-black, grewish colour on plate	Multicelled, matalae with phialides form

Trichodermaspp	Yellowish green on plate	Branch phialides with chlamydosphores
Candida spp	Whitish on petri dish	Multicelledmetalae with pseudohyphae form
Curvulariaspp	Shiny velvet black fluffy growth	Curve septate hyphae with conidia
Fusariumspp	Pink, pluffy with creamy surface around its edges	Septate hyphae with sickle chlamydosphores at the hyphae
Rhizopusspp. Rhodotorulaspp	Long hyphael growth sporulates to black. Pink to red colour	Non-septate hyphae with mycelium bearing terminal sporangioshorescolumella. Pseudohyphae form
Cladosporiumspp	Powdery olivaceous-brown,blackish brown growth on plate	Short condiophoresbranched conidial chain
Cephalosporiumsp	Grey colour on plate	Conidia bearing phialides

Table 4: Morphological and Biochemical Characterization of Bacterial Isolates

Suspected organism	Cell shape	Gram stain	Spore stain	Cat	Ind	Cit	MR	Vp	Ur	Mot	Oxid
Pseudomonas spp.	Rod	-	-	+	-	+	-	-	-	+	+
Bacillus spp.	Rod	+	+	+	-	-	-	-	-	+	+
Staphylococcus spp.	Cocci	+	-	+	-	-	+	-	-	-	+
Streptococcus spp.	Cocci	+	-	-	-	-	+	+	-	-	-
Escherichia coli	Rod	-	-	+	+	-	+	-	-	+	-
Micrococcus spp.	Cocci	+	-	+	-	-	-	-	+	-	+
Klebsiellaspp.	Rod	-	-	+	-	+	-	+	+	-	-
Corynebacteriumspp	Rod	+	-	+	+	+	+	+	-	-	-

Key + present (positive)
- absent (negative)
Cat Catalase
IndIndole
Cit Citrate
MR Methyl red
VpVogesproskauer
Ur Urease
Mot Motility
Oxid Oxidase

Table 5: Fungal Count of Soil Contaminated with Crude Oil and Kerosene

Day		0.0%	1.0%	5.0%	10.0%	15.0%	20%
0	Cr	3.2×10^5	3.0×10^5	2.7×10^5	2.4×10^5	2.1×10^5	1.7×10^5
	Kr	2.8×10^5	2.9×10^5	2.5×10^5	2.2×10^5	2.0×10^5	1.9×10^5
7	Cr	3.8×10^5	5.1×10^5	6.4×10^5	7.2×10^5	7.8×10^5	8.3×10^5
	Kr	3.2×10^5	4.1×10^5	4.7×10^5	5.0×10^5	5.8×10^5	6.3×10
14	Cr	4.2×10^5	6.9×10^5	7.5×10^5	8.1×10^5	9.9×10^5	1.04×10^6
	Kr	4.4×10^5	6.0×10^5	7.2×10^5	8.8×10^5	9.4×10^5	1.02×10^6
21	Cr	4.8×10^5	8.5×10^5	9.7×10^5	1.02×10^5	1.18×10^6	1.26×10^5
	Kr	4.2×10^5	5.3×10^5	6.5×10^5	8.2×10^5	8.9×10^5	9.5×10^5
28	Cr	4.5×10^5	5.6×10^5	7.0×10^5	8.2×10^5	9.8×10^5	1.13×10^6
	Kr	3.9×10^5	4.9×10^5	5.8×10^5	6.4×10^5	6.8×10^5	8.7×10^5

Key Cr Crude oil
Kr Kerosene

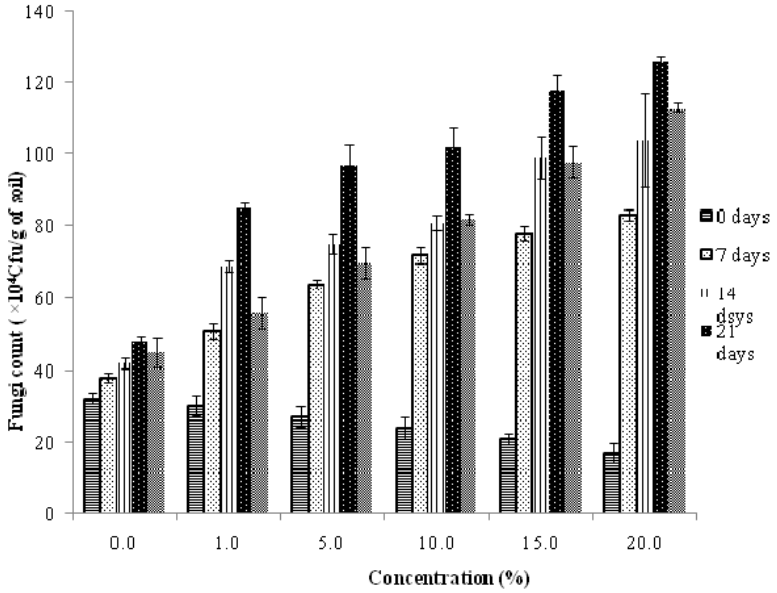

Figure 1: Effect of Crude Oil on the Fungal Population in Soil

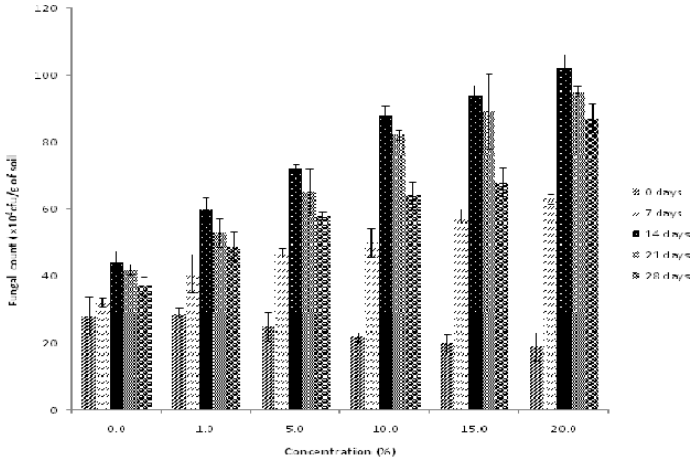

Figure 2: Effect of Kerosene on the Fungal Population in Soil

Bacterial Count in Soil Contaminated with Crude Oil and Kerosene

The data obtained from the average count of bacterial in the soil treated with various concentrations of crude oil gives the following data. The average count of bacterial (colony forming unit /gram) at 0 % crude oil treatment was 3.3×10^7 at 0 day; 3.7×10^7 at 7 days; 4.8×10^7 at 14 days; 4.4×10^7 at 21 days and 3.9 $\times10^7$ at 28 days. For 1 %, 3.6 at 0 day; 4.610^7 at 7 days; 7.7×10^7 at 14 days; 9.2×10^7 at 21 days and 6.8 $\times10^7$ at 28 days. For 5 %, 3.6×10^7 at 0 day; 4.6×10^7 at 7 days; 7.7×10^7 at 14 days; 9.2×10^7 at 21 days and 6.8×10^7 at 28 days. 2.8×10^7 at 0 day; 5.8×10^7 at 7 days; 1.07×10^8 at 14 days; 1.13×10^8 at 21 days and 8.4×10^7 at 28 days for 10 %. 2.1×10^7 at 0 day; 7.4×10^7 at 7 days; 1.28×10^8 at 14 days; 1.30 $\times10^8$ at 21 days and 1.17×10^8 at 28 days for 15 %. 2.0×10^8 at 0 day; 8.0×10^8 at 7 days; 1.33×10^8 at 14 days; 1.43×10^8 at 21 days and 1.26×10^8 at 28 days for 20 % crude oil treatment respectively. The average count of total bacterial (colony forming unit /gram) at 0 % kerosene treatment was 3.4×10^7 at 0 day; 4.0×10^7 at 7 days; 4.6×10^7 at 14 days; 4.0

$\times 10^7$ at 21 days and 3.5×10^7 at 28 days. For 1 %, 3.8×10^7 at 0 day; 4.4×10^7 at 7 days; 8.9×10^7 at 14 days; 4.9×10^7 at 21 days and 4.1×10^7 at 28 days. For 5 %, 2.9×10^7 at 0 day; 5.7×10^7 at 7days; 1.02×10^8 at 14 days; 8.2×10^7 at 21 days and 7.1×10^7 at 28 days. 2.6×10^7 at 0 day; 6.6×10^7 at 7 days; 1.18×10^8 at 14 days; 9.7×10^7 at 21 days and 8.3×10^7 at 28 days for 10 %. 2.3×10^7 at 0 day; 7.2×10^7 at 7 days; 1.25×10^8 at 14 days; 1.01×10^8 at 21 days and 9.7×10^7 at 28 days for 15 %. 2.1×10^7 at 0 day; 8.5×10^7 at 7 days; 1.36×10^8 at 14 days; 1.20×10^8 at 21 days and 1.14×10^8 at 28 days for 20 % crude oil treatment respectively.

Table 6: Bacterial Count of Soil Contaminated with Crude Oil and kerosene

Day	0.0%	1.0%	5.0%	10.0%	15.0%	20%
0 Cr	3.3×10^7	3.6×10^7	2.8×10^7	2.4×10^7	2.1×10^7	2.0×10^7
Kr	3.4×10^7	3.8×10^7	2.9×10^7	2.6×10^7	2.3×10^7	2.1×10^7
7 Cr	3.7×10^7	4.6×10^7	5.8×10^7	6.9×10^7	7.4×10^7	8.0×10^7
Kr	4.0×10^7	4.4×10^7	5.7×10^7	6.0×10^7	7.2×10^7	8.5×10^7
14 Cr	4.8×10^7	7.7×10^7	1.07×10^8	1.21×10^8	1.28×10^8	1.33×10^8
Kr	4.6×10^7	8.9×10^7	1.02×10^8	1.11×10^8	1.25×10^8	1.36×10^8
21 Cr	4.4×10^7	9.2×10^7	1.13×10^8	1.25×10^8	1.30×10^8	1.43×10^8
Kr	4.0×10^7	4.9×10^7	8.2×10^7	9.7×10^7	1.01×10^8	1.20×10^8
28 Cr	3.9×10^7	6.8×10^7	8.4×10^7	1.0×10^8	1.17×10^8	1.26×10^8
Kr	3.5×10^7	4.1×10^7	7.1×10^7	8.3×10^7	9.7×10^7	1.14×10^8

Key Cr Crude oil
Kr Kerosene

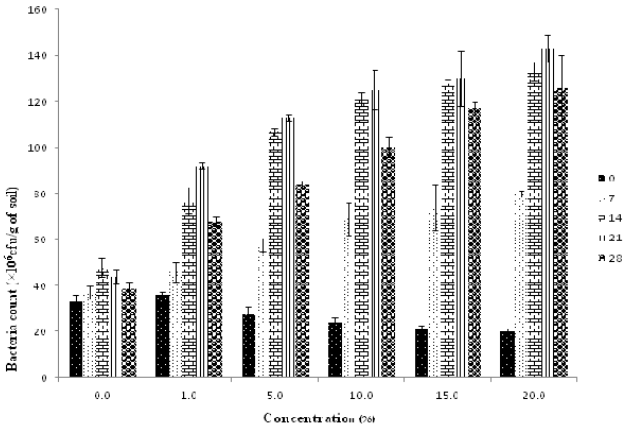

Figure 3: Effect of Crude Oil on the Bacterial Population in the Soil

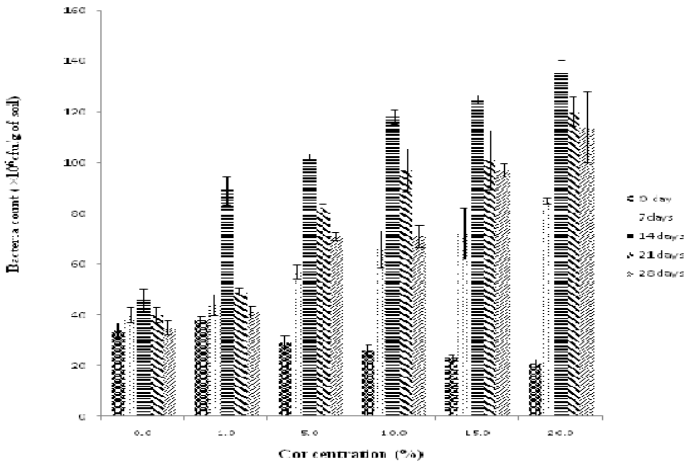

Figure 4: Effect of Kerosene on the Bacterial Population in Soil

Crude Oil and Kerosene Contaminated Soil Effect in Species Richness

Tables below demonstrate occurrence of some fungal and bacterial isolates on crude oil and kerosene contaminated soil causing decrease in species richness. Fungal isolatesTrichodermaspp, Penicilliumspp, Aspergillusspp, Rhizopusspp, Alternariaspp, Candida spp, Curvulariaspp, Fusariumspp, Clasdosporiumspp, Cephalosporiumspp, Rhodotorulaspp, Mucor.Bacterial isolates Pseudomonas spp, Bacillusspp, Staphylococcus spp, Streptococcusspp, Micrococcusspp, Klebsiellaspp, Corynebacteriumspp and Escherichia coli**.**

Table 7: Fungal Isolates Occurrence in 0 % Concentration of Crude Oil Contaminated Soil

Organism	0 day	7 days	14 days	21days	28 days
Aspergillusspp	+	+	+	+	+
Penicilliumspp	+	+	+	+	+
Mucorspp	+	+	+	+	+
Alternariaspp	+	+	+	+	+
Trichodermaspp	+	+	+	+	+
Candida spp	+	+	+	+	+
Curvulariaspp	+	+	+	+	+
Fusariumspp	+	+	+	+	+
Rhizopusspp	+	+	+	+	+
Rhodotorulaspp	+	+	+	+	+
Cladosporiumpp	+	+	+	+	+
Cephalosporiumspp	+	+	+	+	+

Key + present
- absent

Table 8: Fungal Isolates Occurrence in 1 % Concentration of Crude Oil Contaminated Soil

Organism	0 day	7 days	14 days	21days	28 days
Aspergillusspp	+	+	+	+	+
Penicilliumspp	+	+	+	+	+
Mucorspp	+	+	+	+	+
Alternariaspp	+	+	+	+	-
Trichodermaspp	+	+	+	+	-
Candida spp	+	+	+	-	-
Curvulariaspp	+	+	+	+	+
Fusariumspp	+	+	+	-	-

Rhizopusspp	+ + + + + +
Rhodotorulaspp	+ + + + -
Cladosporiumpp	+ + + + + -
Cephalosporiumspp	+ + + + + -

Key + present
- absent

Table 9: Fungal Isolates Occurrence in 20 % Concentration of Crude Oil Contaminated Soil

Organism	0 day 7 days	14 days	21days	28 days
Aspergillusspp	+ + + + +			
Penicilliumspp	+ + + + +			
Mucorspp	+ + + + +			
Alternariaspp	+ - - - -			
Trichodermaspp	+ + - - -			
Candida spp	+ + - - -			
Curvulariaspp	+ + + + -			
Fusariumspp	+ + + + - -			
Rhizopusspp	+ + + + + +			
Rhodotorulaspp	+ + + - -			
Cladosporiumpp	+ + + + - -			
Cephalosporiumspp	+ + + - - -			

Key + present
- absent

Table 10: Fungal Isolates Occurrence in 0 % Concentration of Kerosene Contaminated Soil

Organism	0 day 7 days	14 days	21days	28 days
Aspergillusspp	+ + + + +			
Penicilliumspp	+ + + + +			
Mucorspp	+ + + + +			
Alternariaspp	+ + + + +			
Trichodermaspp	+ + + + +			
Candida spp	+ + + + +			
Curvulariaspp	+ + + + +			
Fusariumspp	+ + + + +			
Rhizopusspp	+ + + + +			
Rhodotorulaspp	+ + + + +			
Cladosporiumpp	+ + + + +			
Cephalosporiumspp	+ + + + +			

Key + present
- absent

Table 11: Fungal Isolates Occurrence in 1 % Concentration of Kerosene Contaminated Soil

Organism	0 day 7 days	14 days	21days	28 days
Aspergillusspp	+ + + + +			
Penicilliumspp	+ + + + +			
Mucorspp	+ + + + +			
Alternariaspp	+ + + - -			
Trichodermaspp	+ + + - -			
Candida spp	+ + + + +			
Curvulariaspp	+ + + + +			
Fusariumspp	+ + + + +			
Rhizopusspp	+ + + + +			
Rhodotorulaspp	+ + + + +			
Cladosporiumpp	+ + + + +			
Cephalosporiumspp	+ + + + +			

Key + present
- absent

Table 12: Fungal Isolates Occurrence in 20 % Concentration of Kerosene Contaminated Soil

Organism	0 day 7 days	14 days	21days	28 days
Aspergillusspp	+	+	+	+
Penicilliumspp	+	+	+	+
Mucorspp	+	+	+	+
Alternariaspp	+	-	-	-
Trichodermaspp	+	-	-	-
Candida spp	+	-	-	-
Curvulariaspp	+	+	-	-
Fusariumspp	+	+	+	-
Rhizopusspp	+	+	+	+
Rhodotorulaspp	+	+	-	-
Cladosporiumpp	+	+	+	+
Cephalosporiumspp	+	+	+	-

Key + present
- absent

Table 13: Baterial Isolates Occurrence in 0 % Concentration of Crude Oil Contaminated Soil

Organism	0 day 7 days	14 days	21days	28 days
Pseudomonas spp	+	+	+	+
Bacillus spp	+	+	+	+
Staphylococcusspp	+	+	+	+
Streptococcusspp	+	+	+	+
Escherichia coli	+	+	+	+
Micrococcus spp	+	+	+	+
Klebsiellaspp	+	+	+	+
Corynebacteriumspp	+	+	+	+

Key + present
- absent

Table 14: Bacterial Isolates Occurrence in 1 % Concentration of Crude Oil Contaminated Soil

Organism	0 day 7 days	14 days	21days	28 days
Pseudomonas spp	+	+	+	+
Bacillus spp	+	+	+	+
Staphylococcusspp	+	+	+	-
Streptococcusspp	+	+	-	-
Escherichia coli	+	+	+	-
Micrococcus spp	+	+	+	+
Klebsiellaspp	+	+	-	-
Corynebacteriumspp	+	+	+	-

Key + present
- absent

Table 15: Bacterial Isolates Occurrence in 20 % Concentration of Crude Oil Contaminated Soil

Organism	0 day 7 days	14 days	21days	28 days
Pseudomonas spp	+	+	+	+
Bacillus spp	+	+	+	+
Staphylococcusspp	+	+	-	-
Streptococcusspp	+	-	-	-

Escherichia coli	+	-	-	-	-
Micrococcus spp	+	+	+	+	-
Klebsiellaspp	+	+	-	-	-
Corynebacteriumspp	+	+	-	-	-

Key + present
- absent

Table 16: Baterial Isolates Occurrence in 0 % Concentration of Kerosene Contaminated Soil

Organism	0 day	7 days	14 days	21days	28 days
Pseudomonas spp	+	+	+	+	+
Bacillus spp	+	+	+	+	+
Staphylococcusspp	+	+	+	+	+
Streptococcusspp	+	+	+	+	+
Escherichia coli	+	+	+	+	+
Micrococcus spp	+	+	+	+	+
Klebsiellaspp	+	+	+	+	-
Corynebacteriumspp	+	+	+	+	+

Key + present
- absent

Table 17: Bacterial Isolates Occurrence in 1 % Concentration of Kerosene Contaminated Soil

Organism	0 day	7 days	14 days	21days	28 days
Pseudomonas spp	+	+	+	+	+
Bacillus spp	+	+	+	+	+
Staphylococcusspp	+	+	+	+	+
Streptococcusspp	+	+	+	+	-
Escherichia coli	+	+	-	-	-
Micrococcus spp	+	+	+	+	+
Klebsiellaspp	+	+	+	-	-
Corynebacteriumspp	+	+	+	+	-

Key + present
- absent

Table 18: Bacterial Isolates Occurrence in 20 % Concentration of Kerosene Contaminated Soil

Organism	0 day	7 days	14 days	21days	28 days
Pseudomonas spp	+	+	+	+	+
Bacillus spp	+	+	+	+	+
Staphylococcusspp	+	+	+	-	-
Streptococcusspp	+	-	-	-	-
Escherichia coli	+	-	-	-	-
Micrococcus spp	+	+	+	+	-
Klebsiellaspp	+	+	-	-	-
Corynebacteriumspp	+	+	-	-	-

Key + present
- absent

A total of twelve fungal isolates were demonstrated in this study and they belong to the genera Aspergillus, Alternaria, Candida, Curvularia, Fusarium, Penicillium, Mucor, Cephalosporium, Trichoderma, Cladosporium, Rhizopus and Rhodotorula and eight bacterial isolates belonging to the genera. Pseudomonas, Bacillus, Staphylococcus, Streptococcus, Escherichia, Micrococcus, Klebsiella, and Corynebacterium.

The statistical analysis of the counts of the total fungi and bacteria in the control and crude oil-polluted soils showed that there was a significant difference ($P < 0.05$) in the average total counts being lowest in the control soil. The order of decreasing average counts of fungi and bacteria in the soils treated with various concentrations of crude oil and kerosene were 20 % > 15% > 10 % > 5 % > 1 % > 0 %. Apart from 24 hrs after pollution, the counts of total fungi were more in polluted soils than in natural soil, which is the control (0 %). The order of decreasing average fungal counts of soils after 24 hours of pollution was 20 % > 15 % > 10 % > 5 % > 1 % > 0 %, which agree with the work of Obire and Anyanwu, (2009) in the results of their study which shows that the addition of crude oil concentrations > 3 % to soils resulted in the selective increase in fungal populations and a reduction of species diversity by the total elimination of certain species.

A sampling at 0, 7, 14 and 21days resulted in the multiplicity of the bacteria and fungi load and reduction in species richnessin the soil treated with crude oil and decline in microbial count observed in polluted soils toward 28 days of the incubation period. A sampling at 0, 7, 14 days resulted in the multiplicity of the bacteria and fungi load and reduction in species richness in the soil treated with kerosene oil and decline in microbial count observed in polluted soils toward 21 and 28 days of the incubation period. The increase in microbial population in days may be explained by the fact that when the crude oil and kerosene were freshly applied to the soil, it causes toxicity to the soil microorganisms, damage to the soil biota., this coincides with the work of Seghers*et al*., 2003; Hofman*et al*., 2004; Gramss*et al*., 1998; Okpokwasili and Okorie, 1988. Seghers*et al*., 2003Hofman*et al*. 2004; Van-Dorst*et al.*, 2014; Huo*et al.*, 2018 revealed from their work that the number of soil microorganisms increases in petroleum hydrocarbon - polluted soils, species richness decreases over time. Gramss*et al*., 1998; demonstrated that when soil is polluted with PHC, reduction in soil microorganisms species is observed especially in soils that have not been previously polluted. A study by Okpokwasili and Okorie, 1988 demonstrated the Biodeterioration potentials of microorganism isolated from car engine lubricating oil.

Varjani and Upasani, 2017 reported that many environmental factors such as temperature, nutrients, electron acceptors and substrates play vital roles in bioremediation and influence biodegradation reactions. Petroleum derivatives may decrease the exchange of oxygen between the soil and the atmosphere, thus decreasing the availability of oxygen for microbiota. Lower content of oxygen in the soil atmosphere lead to alteration of the redox state (Pena, 2007) thus yielding more reduced conditions. The immediate effect of these changes would be a decrease in an aerobic microorganism (Pena, 2007). Another effect of lower oxygen content would be an alteration in microbial communities, leading to changes in the relationships between diverse groups of microbiota (Santos, 2012; Megharaj, 2000).

The adaptability of the microorganism to the polluted soil led to a rapid increase in the microbial population of crude oil and kerosene utilizing bacteria and fungi. Sampling at 21 days in different concentration of crude oil recorded a significant increase in microbial counts, thus this was the period with the highest microbial counts both in bacteria and fungi, Sampling at 14 days in different concentration of kerosene recorded a significant increase in microbial counts, thus this was the period with the highest microbial counts both in bacteria and fungi. Hofman *et al*. 2004 recorded the number of soil microorganism’s increase in PHC-polluted soils over time. The effects of the presence of hydrocarbons lead to the death of microbial populations that are sensitive to these contaminants (Serrano *et al.,* 2009; Margesin*et al.*, 2000; Labud, 2007; Serrano, 2008; Tao *et al.*, 2017). A large number of hydrocarbon-degrading bacteria agree with the study of Hazen *et al.*, 2010; Yang *et al.*, 2015 who report that there is a large number of hydrocarbon-degrading bacteria in oil-rich environments, such as oil spill areas and oil reservoirs, and that their abundance and quantity are closely related to the types of petroleum hydrocarbons and the surrounding environmental factors (Fuentes et al., 2015; Varjani and Gnansounou, 2017)

The significant increase observed in this study stems from the fact that as most biodegraders recovered from the initial shock, they multiply. This agrees with the work of Ekpo (2006) who worked on the biodegradation of bonny light and bonny medium crude oil, noted that the initial outcome of a natural microbial population in contact with petroleum hydrocarbon is most often a reduction in the microbial biomass followed by an increase in bio-degraders. Gramss*et al.*, (1998) reported that the reduction in the number of microorganism in hydrocarbon polluted soil is followed by a rapid increase in the number of microorganisms that are capable of degrading the contaminants.Guerra *et al.*, 2018; Xu*et al.*,2018, observed an ability of a microorganism to biodegrade petroleum oil is associated with the concentration and composition of hydrocarbons. The fungal and bacterial isolates have the ability to tolerant crude oil and kerosene pollution on various soil, Thus microorganisms having great potential for bioremediation.

Extremely high levels of petroleum hydrocarbons strongly inhibit bacterial growth, resulting in poor biodegradation efficiency and even the death of the bacteria (Ma *et al.*, 2015; Labud*et al.*, 2007). The decline in microbial count observed in polluted soils toward the end of the incubation period may be as a result of nutrient exhaustion and the introduction of toxic metabolites (McGill and Nyborg, 1975). Contamination of soil is a particularly serious problem because of the impact that it has on soil functioning, and on the whole ecosystem. Agricultural soils, which are continually exploited to produce food and fodder, are particularly sensitive to contamination as agricultural soils generally display poor resilience, that is they are incapable of recovering from any type of aggression, and any type of contamination, The effect of crude oil and kerosene brought about alterations to soil functioning.

CONCLUSIONS

The results of this study show decreasing order of occurrence of a variety of fungal and bacterial genera thus alters the biochemical functions in the soil system and affects the soil quality, soil stability, soil property, microbial activities, and agricultural production. However, some microorganisms were able to adapt and grow under various extreme conditions and show a high level of tolerance for crude oil and kerosene tested which makes them attractive potential candidates for further investigations regarding their ability to remove hydrocarbon from the soil. It may be a good option for bioremediation of soil since it is regarded as an eco-friendly and efficient. From this study, the use of crude oil and kerosene were poorly disposed. Therefore, there should be regulations on the disposal of petroleum product on soil and maintenance of pipeline to avoid environmental degradation.

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