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The Microbiological and Physicochemical Properties of Some Crude Oil Contaminated and Uncontaminated Agricultural Soils in Ondo State, Nigeria

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Abstract

This study was undertaken to assess the microbiological and physico-chemical properties of some crude oil contaminated and uncontaminated agricultural soils in Ondo State, Nigeria. The identities of the crude oil degraders and some physicochemical properties of the soils collected from Awoye, Orioke-Iwamimo, Igodan-Lisa, Oba-Ile and Idoani all in Ondo State, Nigeria were studied using conventional microbiological, standard physical and chemical analytical techniques. The identities of the crude oil degrading microbes include *Klebsiella pneumoniae*, *Klebsiella edwardsii*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Bacillus megaterium*, *Alcaligenes faecalis*, *Proteus mirabilis*, *Acinetobacter mallei*, *Cephalosporium* species, *Trichoderma* species, *Fusarium* species, *Aspergillus* species, *Pullularia pullulans*, *Kodamaea ohmeri*, *Scopulariopsis brevicaulis, Paecilomyces variotii*, *Candida parapsilopsis*, *Mucor mucedo,* and *Trichophyton menragrophytes*. The percentage crude oil degrading microbes ranged 23.00 - 28.74% and 12.09 – 90.04% respectively for bacteria and fungi. The Awoye and Orioke-Iwamimo soils contained the highest amounts of clay (40.07% and 39.67%) and silt (40.35% and 39.31%) respectively. Sand contents of the samples ranged from 18.66 - 68.36%. Organic carbon (4.70%), Organic matter (8.03%) and Total nitrogen (2.28%) were highest in Idoani while moisture contents were highest in Awoye (54. 12%) and Orioke-Iwamimo (54.80%) soil samples against the 8.36 – 9.38% for the other samples. The pH of the samples ranged 6.7 - 8.2. The total petroleum hydrocarbon contents of 8.59 - 13.27mg/kg were recorded for samples. Results also revealed varying amount of Copper, Lead, Nickel, Vanadium and Cadmium. This research showed that the soils vary in their physico-chemical characteristics and as well contained a wide array of crude oil degrading microbes that can be used in seeding of polluted soils for bioremediation.

Keywords: Microorganisms, crude oil degraders, physicochemical properties, Ondo State, bacteria, fungi, bioremediation.

INTRODUCTION

Soil can be defined in many ways to suit different professions and purposes. To the agriculturist, soil is defined as the medium for crop growth, anchorage for plants; providing nutrients, water and air on which plants depend (Ibitoye, 2006). Soil also provides habitat for microflora and microfauna. Soil is made of four components; sand, silt, clay and humus (decayed organic materials). Sand is important for keeping the soil loose, aerated and well drained. Clay minerals hold water and nutrients in the soil just loosely enough to allow plant roots to absorb them. The humus component provides the bulk of the soil's fertility (European Commission, 2015).

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The major source of energy for fulfilling the energy requirement to drive the global economy is crude oil (Odeyemi, 2014). Agriculture is the main occupation of the people of Ondo State and Nigeria in general. The people depend solely on sales from their farms for food and economy. Pollution of the environment with crude oil due to human activities of exploration, exploitation, distribution, usage of crude oil and its derivatives has deleterious effects on the entire ecosystem and all forms of living organisms supported by the environment because of the wide array of toxic compounds and their concentrations (Franco et al., 2004; Olabemiwo et al., 2011; Odeyemi, 2014), causing alterations in soil microbiological and physicochemical properties (Ijah and Antai, 2003; Agamuthu and Dadrasnia, 2013; Odeyemi, 2014). In petroleum producing region, crude oil can sterilize the soils and prevent crop growth for varying periods of time (Atlas and Bartha, 1992), reduces soil's fertility such that most of the nutrients are no longer available for plant and crop utilization (Abii and Nwosu, 2009), creating nutritional imbalances(especially of carbon – nitrogen ratio) at the spilled sites (Chorom et al., 2010) and therefore renders the agricultural lands less productive (Wokocha et al., 2011; Etuk et al.,2013) with overall implications on the economy of the people residing in the crude oil polluted area due to high incidence of unemployment and poverty rates (Etuk et al., 2013).

The socio – economic as well as environmental values of soil demands that soil must be protected, maintained and sustained free from contamination. Bioremediation as a contaminant removal strategy relies on the diverse metabolic activities of microorganisms to degrade or remove organic pollutants from the environment. Among the parameters that appear to be important in bioremediation include the properties of the hydrocarbon to be degraded i.e the composition and inherent biodegradability of the petroleum hydrocarbon pollutant, soil Ph, types and population of native microorganisms, oxygen concentration, temperature, soil moisture, soil texture, nutrient availability (Sabate *et al.,* 2004; Das and Chandran, 2011; Jain et al., 2011; Odeyemi, 2014).

Farag and Soliman, (2011) reported that degradation of hydrocarbon by natural population of microorganisms in polluted areas is the main process acting in the restoration of hydrocarbon polluted soil. Microbial communities present in previously crude oil contaminated soils are stimulated to metabolize petroleum hydrocarbon at greater rates than soil microbial communities found in uncontaminated soils. This is because communities exposed to hydrocarbons become adapted (acclimatized), exhibiting selective enrichment and genetic changes (Odeyemi, 2014). The nutrient status of soil has direct impact on microbial activity and biodegradation (Jain *et al.,* 2011). Significantly, nitrogen, phosphorous, sulphur and in some cases iron are known to limit hydrocarbon degradation since the contaminant itself is a carbon source (Das and Chandran, 2011, Odeyemi, 2014). This therefore implies that microbial growth may be limited by several elements at the same time and addition or combination of nutrients can enhance biodegradation. Soil moisture is another important parameter in determining the rate of biodegradation of petroleum hydrocarbons. Microbes live in the interstitial water of soil pores and the lower the amount of water, the smaller the number of microbes and thus, slow removal rate through biodegradation (Jain et al., 2011). As the water solubility of many solid hydrocarbon is very low, transport limitation can cause recalcitrance of an otherwise degradable hydrocarbon. Microbial activity in soil is generally greatest at water contents ranging between 50 – 80% of the maximum holding capacity (Odeyemi, 2014). Soil Ph is a measure of the acidity or alkalinity of the soil. The optimum Ph for hydrocarbon biodegradation is about neutral Ph 6 – 8 (Odeyemi, 2014). Biodegradation in an acidic soil of Ph 4.5 could be doubled by liming to Ph 7.4 (Odeyemi, 2014). Soil texture including those that restrict the mass transfer of the compound to the microorganisms such as clay and organic matter play significant roles in the degradation of compounds (Ralebitso et al., 2002; Odeyemi; 2014). Nackles and Ray (2002) stated that biodegradation will occur in all soil types even though some may need additives or special care or equipment. Clay soils may need to be amended with bulking agents in order to improve oxygen transport. Sandy soils may need to be amended with organic matter to improve the soil water holding capacity.

It is therefore pertinent to be adequately equipped with knowledge of the microbiological and physicochemical status of the soil in order to be able to develop and adopt appropriate strategy to optimize degradation processes so as to improve agricultural yields and hence the livelihood of people living in risk area of crude oil pollution. Therefore, this research aimed at (i) isolation and identification of crude oil degrading microorganisms associated with crude oil contaminated and uncontaminated agricultural soils in Ondo State. (ii). Evaluation of the physicochemical properties of the soils.

MATERIALS AND METHODS

Sample Collection

The crude oil-contaminated soil samples used in this study were collected from Awoye (5^o 59' 0'' N, 4^o 55' 0''E) and Orioke-Iwamimo(6^o11' 0''N, 4^o 41 '0'' E) in Ilaje Local Government Area of Ondo State. These coastline communities had witnessed over thirty four (34) regular crude oil spill of varying quantities in the last few years. The uncontaminated soil samples were collected from Igodan-Lisa (6^o 27' 0''N, 4^o 47'0''E), Oba-Ile (7^o 16' 0''N, 5^o 15' 0''E) and Idoani (7^o 17' 0''N, 5^o 52'0''E), all in Ondo State, Nigeria. These samples have no history of previous exposure to crude oil contamination. Each soil was collected using the hand auger at depth of 15-20cm into sterile black cellophane bags. The samples were then itself is a carbon source (Das and Chandran, 2011; Odeyemi, 2014) partially air-dried at 28±2^{o}C to allow for sieving to uniform consistency with 2mm sieve.

Table 1. Total heterotrophic and crude oil degrading bacteria in soil samples

Sample	Total heterotrophic bacteria (cfu/g)	Crude oil degrading bacteria (cfu/g)	% crude oil degrading bacteria
Awoye	$2.07 \times 10^{9} \pm 6.67 \times 10^{7a}$	$5.71 \times 10^{8} \pm 5.77 \times 10^{5a}$	27.59
Orioke-Iwamimo	$5.07 \times 10^{9} \pm 6.67 \times 10^{7b}$	$1.39 \times 10^{9} \pm 8.80 \times 10^{6b}$	27.42
Igodan-Lisa	$7.10 \times 10^{10} \pm 5.77 \times 10^{8e}$	$1.78 \times 10^{10} \pm 8.82 \times 10^{7d}$	25.07
Oba-Ile	$6.13 \times 10^{9} \pm 8.82 \times 10^{7c}$	$1.41 \times 10^{9} \pm 3.33 \times 10^{6b}$	23.00
Idoani	$1.83 \times 10^{10} \pm 3.33 \times 10^{8d}$	$5.26 \times 10^{9} \pm 1.15 \times 10^{7c}$	28.74

Microbological analysis of soil samples

i). Enumeration and isolation of microorganisms

The methods described by Onifade *et al*., 2007; Onuoha *et al.,* 2011; Omotayo *et al* ., 2012; Ikuesan *et al*., 2015 were used. Nutrient Agar (NA) and Malt Extract Agar (MEA) were used for the isolation of bacteria and fungi respectively. The Mineral Salt Medium (MSM) used for the cultivation of crude oil degrading microbes was Bushnell-Hass broth incorporated with 1.5% agar (for bacteria), 1.2% agar (for fungi), 2.4% NaCl for samples collected from Awoye and Orioke- Iwamimo (salty environments). The media were also fortified with fungisol (10mg/lt) for bacteria and 50mg/Lt of streptomycin for fungi after sterilization. Crude oil (2%) sterilized using 0.45µm Millipore filter served as carbon source. The pH of the medium was adjusted to 7.2 and 5.6 respectively for bacteria and fungi estimation. The MS-oil medium for crude oil degrading bacteria and crude oil degrading fungi were then incubated at 28±2^{o}C respectively for 14 days and 21days.

The colonies which developed on the plates were counts in which the number of colonies 30-300 (Odokuma and Dickson, 2003) and its triplicate for each sample was selected. The averaged count was then multiplied by the dilution factor at that dilution and expressed as colony forming unit (cfu/g) or spore forming unit (sfu/g) per gramme of sample for bacteria and fungi respectively. The percentage of crude oil degraders relative to the heterotrophic load for each sample was calculated.

ii). Characterization and Identification of microbial isolates

Isolates were purified by repeated streaking on fresh agar medium. Characterization of isolates were based on the colonial and cell morphology and biochemical tests including sugar fermentation. The data obtained were compared with standards obtainable in Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994). Fungal isolates were characterized and identified by cultural feature and microscopic observation in lacto-phenol cotton blue. Onions *et al*., (1981) and Barnett and Hunter, (1983) were used for the identification of fungi.

Analysis of soil physicochemical parameters:

i). Soil texture

Soil structural composition was analyzed by the hydrometry method (Ekpo and Ebeagwu, 2009) using sodium hydroxide as dispersing agent.

ii). Moisture content

The weight loss method described by Ijah and Abioye (2003); Osuji and Nwoye (2007).

iii). Soil pH

This was measured by the glass electrode pH meter (Jenway 3051) method in 1:1w/v soil/water suspension, which was standardized at pH 7.0 using phosphate buffer solution (Akubugwo *et al*., 2009).

iv). Organic matter (OM) and Organic carbon (OC)

These were determined by the dichromate oxidation method of Walkley-Black as described by Osuji and Nwoye, (2007); Akubugwo *et al*.,(2009); Ekpo and Ebeagwu,(2009).

v). Total nitrogen and available phosphorous

Total nitrogen was determined by the macro-kjeldahl digestion method (AOAC, 1999) while available phosphorous were assessed by spectrophotometry method (Chopra and Kanwar, 1998).

vi). Total petroleum hydrocarbon (THP) and Heavy metal content

Gas chromatographic method was adapted to measure total petroleum hydrocarbon (Salam *et al*., 2011). The heavy metals of the soil samples were determined using the atomic absorption spectrophotometer.

Statistical analysis

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

RESULTS

Microbiological analysis of soil samples

(i). Enumeration of microorganisms

Table 2. Total heterotrophic and crude oil degrading fungi in soil samples

Sample	Total heterotrophic Fungi (sfu/g)	Crude oil degrading fungi (sfu/g)	% crude oil degrading fungi
Awoye	$3.67 \times 10^{3} \pm 3.33 \times 10^{2a}$	$2.33 \times 10^{3} \pm 5.77 \times 10^{2a}$	63.49
Orioke-Iwamimo	$1.10 \times 10^{5} \pm 1.00 \times 10^{4a}$	$1.33 \times 10^{4} \pm 5.77 \times 10^{3a}$	12.09
Igodan-Lisa	$1.17 \times 10^{6} \pm 8.82 \times 10^{4b}$	$5.0 \times 10^{5} \pm 5.77 \times 10^{4b}$	42.74
Oba-Ile	$4.17 \times 10^{3} \pm 0.88 \times 10^{2a}$	$2.33 \times 10^{3} \pm 3.33 \times 10^{2a}$	55.88
Idoani	$7.03 \times 10^{3} \pm 0.57 \times 10^{2a}$	$6.33 \times 10^{3} \pm 3.33 \times 10^{2a}$	90.04

Table 3. Identities of crude oil degrading microorganisms associated with the soil and cow dung

Sample	Microorganisms	
	Bacteria	**Fungi**
Awoye	*Klebsiella pneumoniae* *Pseudomonas aeruginosa* *Alcaligenes faecalis* *Enterobacter* species *Bacillus subtilis* *Klebsiella edwardsii* *Brevundimonas diminuta* *Bacillus megaterium*	*Trichoderma* species *Cephalosporium* species *Fusarium* species
Orioke- Iwamimo	*Pseudomonas aeruginosa* *Flavobacteriun* species *Bacillus aryabhattai* *Klebsiella pneumoniae* *Klebsiella edwardsii* *Proteus mirabilis*	*Trichoderma* species *Mucor mucedo* *Aspergillus glaucus* *Pullularia pullulans* *Kodamaea ohmeri*
Igodan- Lisa	*Pseudomonas aeruginosa* *Bacillus subtilis* *Klebsiella edwardsii* *Klebsiella pneumoniae* *Alcaligenes faecalis*	*Aspergillus flavus* *Trichoderma* species *Aspergillus flavus* *Trichophyton menragrophytes* *Cephalosporium* species *Fusarium* species *Gliocladium* species *Scopulariopsis brevicaulis*
Oba- Ile	*Acinetobacter mallei,* *Klebsiella edwardsii,* *Klebsiella pneumoniae* *Bacillus megaterium* *Pseudomonas aeruginosa*	*Pullularia pullulans,* *Aspergillus fumigatus* *Paecilomyces variotii;* *Candida parapsilosis* *Kodamaea ohmeri*
Idoani	*Pseudomonas aeruginosa* *Klebsiella edwardsii* *Klebsiella rhinoscleromatis* *Klebsiella pneumoniae* *Bacillus subtilis* *Bacillus megaterium* *Bacillus aryabhattai*	*Mucor mucedo* *Fusarium* species *Trichoderma* species *Ceratobasidium* species *Aspergillus aculeatinus*

Note: Detailed characteristics not shown

Table 4(a). Physicochemical Characteristics of Soils from Different Sample Locations in Ondo State

Sample	% Sand	% Silt	% Clay	% Organic carbon	% Organic matter	% Moisture content	% Available phosphorus	% Total Nitrogen	pH	TPH (mg/kg)
Awoye	18.66±0.01^{a}	40.35±0.03^{f}	40.07±0.07^{f}	0.29±0.01^{a}	0.44±0.01^{a}	54.12±0.01^{d}	9.16±0.00^{a}	0.91±0.01^{a}	7.2±0.00^{c}	9.07±0.01^{b}
Orioke Iwamimo	19.31±0.01^{b}	39.31±0.02^{e}	39.67±0.01^{e}	0.54±0.01^{b}	0.91±0.01^{b}	54.80±0.06^{e}	10.80±0.00^{b}	1.27±0.00^{b}	8.2±0.03^{d}	8.59±0.00^{a}
Igodan-Lisa	51.32±0.01^{c}	36.34±0.01^{d}	13.21±0.11^{c}	4.69±0.01^{d}	7.99±0.01^{d}	8.36±0.01^{a}	26.14±0.01^{e}	1.63±0.00^{c}	7.0±0.00^{b}	13.27±0.00^{f}
Oba-Ile	67.35±0.01^{d}	20.35±0.01^{c}	12.67±0.01^{b}	3.91±0.01^{c}	6.63±0.01^{c}	8.50±0.00^{b}	17.60±0.00^{d}	1.93±0.01^{d}	6.7±0.03^{a}	10.57±0.00^{c}
Idoani	68.36±0.02^{e}	15.68±0.01^{a}	14.66±0.00^{d}	4.70±0.00^{d}	8.03±0.01^{e}	9.38±0.00^{c}	11.41±0.01^{c}	2.28±0.01^{e}	7.0±0.03^{b}	11.96±0.01^{e}

Table 4(b): Heavy Metal Composition Characteristics of Soil Samples from Different Sources

Sample	Copper (mg/kg)	Lead (mg/kg)	Nickel (mg/kg)	Vanadium (mg/kg)	Cadmium (mg/kg)
Awoye	6.38 ± 0.01^{b}	12.36 ± 0.01^{a}	6.03 ± 0.01^{b}	2.19 ± 0.003^{b}	0.17 ± 0.003^{e}
Orioke-Iwamimo	5.41 ± 0.01^{a}	10.47 ± 0.01^{e}	4.28 ± 0.01^{e}	1.44 ± 0.03^{c}	0.18 ± 0.003^{c}
Igodan- Lisa	6.23 ± 0.01^{a}	14.37 ± 0.01^{b}	5.75 ± 0.01^{c}	1.89 ± 0.003^{e}	0.15 ± 0.003^{b}
Oba-Ile	4.61 ± 0.01^{c}	11.46 ± 0.02^{d}	5.42 ± 0.01^{a}	2.90 ± 0.003^{a}	0.14 ± 0.003^{a}
Idoani	5.76 ± 0.01	12.68 ± 0.01	3.95 ± 0.01	1.39 ± 0.003	0.12 ± 0.003

Results also revealed that samples had varying amount of available Phosphorus, Copper, Lead, Nickel, Vanadium and Cadmium.

Table 1 shows the population of Total Heterotrophic Bacteria (THB) and Crude oil Degrading Bacteria (CDB) while table 2 shows the Total Heterotrophic Fungi (THF) and Crude oil Degrading Fungi (CDF). The THB and THF counts in the five samples ranged 2.07 x 10^{9} – 7.10 x 10^{10}cfu/g and 3.67 x 10^{3}– 1.17 x 10^{6}sfu/g respectively The counts of THB and THF of 7.10 x 10^{10} cfu/g and 1.17 x 10^{6} sfu/g respectively were highest in the Igodan-Lisa soil sample compared with other samples. The crude oil contaminated Awoye soil sample had the least heterotrophic bacterial counts of 2.07 x 10^{9} cfu/g and 3.67 x 10^{3} sfu/g of THF. Results also revealed lowest counts of crude oil degrading bacteria (CDB) and crude oil degrading fungi (CDF) in the Awoye soil. The crude oil degrading bacteria and fungi in samples ranged 5.71 x 10^{8} – 1.78 x 10^{10} cfu/g and 2.33 x 10^{3} – 5.00 x 10^{5} sfu/g. Results also revealed that the Idoani sample had the highest percentage of crude oil degrading bacteria (28.74%) and crude oil degrading fungi (90.04%).

(ii) Characterization and identification of crude oil degrading microorganisms

On the basis of cultural, morphological and biochemical characteristics, the identities of the intrinsic crude oil degrading microorganisms in the samples are shown in table 3. Results revealed that the crude oil degrading bacterial isolates obtained in this study are predominantly gram negative, *Bacillus* species being the only gram positive bacterial isolates found common to all the samples investigated. Similarly, except for *Candida parapsilopsis* and *Kodamaea ohmeri* which are yeasts the other fungal isolates are moulds.

(iii). Physicochemical properties of soil samples:

Tables 4(a and b) show some physicochemical properties of soil samples. Results revealed that the Awoye and Orioke-Iwamimo soils contained the highest amount of clay (40.07% and 39.67%) and silt (40.35% and 39.31%). The Awoye and Orioke-Iwamimo soils are classified as clay while the other soils are sandy according to their structural composition. The Igodan - Lisa, Oba-Ile and Idoani samples were revealed to have high percentage of sand which ranged 51.32 - 68.36%. Organic carbon (OC), Organic matter (OM) and Total nitrogen (TN) were highest in the Idoani sample. There is no significant difference at P≤0.05 confidence interval in the organic carbon content of Idoani and Igodan- Lisa soils. The OC, OM and TN contents ranged between 0.29 - 4.70%, 0.44

- 8.03% and 0.91 - 2.28% respectively. The moisture contents were highest in Orioke-Iwamimo and Awoye samples (54.80% and 54.12%) respectively. The soil from Oba-Ile was found to be slightly acidic (pH 6.7) compared with the other samples with neutral or alkaline pH in the range of 7.0 - 8.2. The TPH contents of samples investigated by gas chromatography revealed that the samples had varying amount of Total Petroleum Hydrocarbon (TPH) which ranged 8.59mg/kg – 13.27mg/kg. The least amount of TPH was obtained from the Orioke-Iwamimo sample while the Igodan- Lisa sample had the highest amount.

DISCUSSION

The findings in this research indicated that the environment from which samples were collected for this study vary in their microbiological and physico-chemical properties. They also contain considerable number and diverse types of heterotrophic and crude oil degrading bacteria and fungi. The counts of total heterotrophic microbes were higher compared to the crude oil degrading microbial population. Naturally, hydrocarbon degrading bacteria and fungi are heterotrophs. Therefore, the environment is expected to have more heterotrophs than crude oil degraders which are subpopulation. The proportion of hydrocarbon degraders 23.00 - 28.74% and 12.09 - 90.04% obtained in this study respectively for bacteria and fungi is suggestive of the fact that apart from the Awoye and Orioke-Iwamimo soil samples, which have had known history of previous regular crude oil pollution of varying magnitude, all the other samples may as well have experienced contamination of crude oil or other forms of hydrocarbon pollution. This assertion is in agreement with the report of Rahman *et al*., (2002) which indicated that in unpolluted ecosystem, hydrocarbon utilizers generally constitute about 0.1% of the microbial community while in polluted systems, they can constitute up to 100% of the viable microorganisms. The high counts of petroleum hydrocarbon degrading microbes in samples is therefore suggestive of previous exposure to crude oil which boost the supply of carbon in the soils hence it favours the growth of these microbes. Atlas (1981) reported that the microbial population of hydrocarbon degrading microorganisms in an ecosystem quantitatively reflect the degree or extent of exposure of that ecosystem to hydrocarbon contamination. Similarly, Rahman *et al*., (2002) reported that the population level of hydrocarbon utilizers and their population within the microbial community appear to be a sensitive index of environmental exposure to hydrocarbons.

Each of the soil samples contained diverse genera of bacteria and fungi. The crude oil degrading microorganisms isolated in this study which include *Pseudomonas aeruginosa*, *Alcaligenes faecalis*, *Bacillus subtilis*, *Enterobacter* species, *Flavobacterium* species, *Proteus mirabilis*, *Cephalosporium* species, *Trichoderma* species, *Aspergillus* species, *Fusarium* species and *Paecilomyces* were among those earlier isolated and identified by many researchers like Ijah and Abioye (2003); Ajayi *et al*., (2008); Das and Chandran (2011); Omotayo *et al*.,(2012); Odeyemi, (2014). These microorganisms are suggestively adapted for survival and proliferation in the polluted and unpolluted environment. The presence of both crude oil degrading bacteria and fungi from the samples is indicative of their implication in the degradation of crude oil and therefore suggestive of their potentials in bioremediation of crude oil polluted agricultural soils. The bacterial isolates obtained in this study belong to both the gram positive and gram negative groups. These findings corroborate the report of Salam *et al*., (2011) that both gram negative and positive bacteria have been implicated in the mineralization of hydrocarbon pollutants. This result agrees with the results obtained by Cappello *et al*.,(2007); Van Beilen and Funhoff (2007); Dasgupta *et al*., (2013) that the ability to degrade or utilize hydrocarbon substrate is exhibited by a wide variety of bacterial genera or diverse indigenous microbial population (Omotayo *et al*.,2011) and are widely distributed in oil polluted as well as pristine soils (Malakootian *et al*., 2009; Abdulsalam and Omale 2009; Abdulsalam *et al*., 2011). The gram negative bacteria however predominate in all the samples. This implies the dominance of gram positive bacteria in bioremediation of crude oil polluted soils in agreement with the report of Kaplan and Kitts (2004) that gram positive bacteria if detected in bioremediation are never diverse and dominant. Worthy of note here is that *Klebsiella edwardsii*, *Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Bacillus* species were obtained in all the soils samples used in this study. This probably suggests that these organisms are able to adapt to many different hydrocarbons and varying environmental parameters since samples were collected from different locations. So, the ability to isolate high number of certain crude oil degrading microorganisms from these environments is suggestive that those microorganisms are the most active degraders in that environment and can be used in the bioremediation of petroleum oil contaminated sites.

The baseline physico-chemical characteristics of the samples in terms of texture, pH, content of moisture, organic carbon, organic matter, available phosphorous, total nitrogen, total petroleum hydrocarbon etc. differ from one location to the other (tables 4a and 4b). All these parameters have roles to play in the growth and survival of native microorganisms. Significantly, the pH values (6.7 - 8.2) obtained in the study for soil samples favoured the growth and proliferation of bacteria and fungi, since these microbes grow over a wide range of pH. Nackles and Ray (2002) reported that bacteria survive better in the pH range of 6.5 - 8.5 and yeasts and molds thrive better at pH range of 4.5 - 5.3. The soils had lower nitrogen

(0.91 - 2.28%) and phosphorous (9.16% - 26.14%). There is no significant difference ($P \leq 0.05$) in the pH and organic carbon contents of Igodan- Lisa and Idoani soil samples. The high clay content of the Awoye and Orioke - Iwamimo soil samples suggests that these soils may not be good for agricultural purposes or otherwise may require bulking agents to enhance bioremediation. The high moisture content of the Awoye and Orioke - Iwamimo soils might reduce microbial activities not as a result of the water itself but by the indirect hinderance to the movement of air which would reduce oxygen supply (Osuji and Nwoye, 2007). Interestingly, the Igodan- Lisa, Oba-Ile and Idoani soil samples considered to be uncontaminated with crude oil had TPH in relatively higher amount (10.57-13.27mg/kg) than the Awoye (9.07mg/kg) and Orioke–Iwamimo (8.59mg/kg) soil samples with regular occurrence of crude oil spill. This implies that agricultural lands of Igodan- Lisa, Oba-Ile and Idoani are undesirably exposed to petroleum hydrocarbons or their derivatives or other forms of hydrocarbon.

CONCLUSION

The quality of life in an environment depends largely on the overall quality of the environment. The remediation of petroleum contaminated soil is a real problem of global interest as it threatens all forms of life dependent on the environment. The proportion of crude oil degrading microbes and the total petroleum hydrocarbon results from this study indicated that arable agricultural soils are consciously or unconsciously exposed to petroleum hydrocarbon contamination with its attendant negative effects on agricultural activities and the livelihood of the people residing in the area. These may be due exposure resulting from indiscriminate discharges, unguided activities and use of petroleum hydrocarbons and its derivatives. The microorganisms isolated and identified in this research when produced and harvested in large biomass can be applied singly or as a consortium to enhance the degradation of crude oil polluted agricultural soils.

RECOMMENDATION

Informed knowledge of bioremediation processes provides a great deal of ecological advantage that depends on the native or exogenous microbial population to degrade or mineralize the contaminant petroleum hydrocarbon. In the future, the efficiency of these microbial isolates and their optimum storage conditions as well as their hydrocarbon specificities as crude oil degraders should be evaluated. This will undoubtedly assist in developing inoculum with high activity to enhance the bioremediation of polluted agricultural soils in Nigeria, especially in the Niger- Delta region where crude oil spill is an environmental menace of great concern.

One of the considerations critical for returning environmentally polluted soils to its pristine state is a thorough knowledge of the impact of oil pollution and the technological parameters for its elimination. Therefore, the effects of varying concentrations of crude oil on the population of crude oil degrading microbes and physico-chemical properties of soil is proposed for future research in order to develop appropriate strategy that will curb themenace of crude oil pollution of agricultural sites.

Appropriate regulatory laws should be enacted and enforced to control the use and discharge of organic pollutants into the environment in order to keep a healthy environment, return the people to their traditional occupation and improve the livelihood of people resident in the risk areas of crude oil pollution.

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