Assessment of Biodegradation Potential on Different Soil Particles

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Abstract: An explosion in the world's population has led to an increase in the demand for crude oil and its products resulting in an increased environmental pollution and thus leading to loss in biodiversity. Environmental reclamation by natural technique is believed to be eco-friendly and cost effective. This research investigated the biodegradation of crude oil supported on different soil particle sizes. Soil samples were randomly collected from different locations within Botanical garden of BUK and sieved into four (4) different particle sizes (0.6mm, 0.3mm, 0.15mm and 0.075mm) 200g of each of the sieved soil particle sizes were weighed and introduced into masonjar bottle. These were then uniformly contaminated with 60ml of crude oil. The set up were replicated in triplicate with each having two (2) controls. They were incubated aerobically at 37^{o}C for 56 days. Enumeration of total aerobic heterotrophic bacteria (TAHB) was done on Nutrient agar (NA) and hydrocarbon utilizing bacteria (HUB) on Bushnell'Haas medium (BHM) supplemented with crude oil. The bacterial isolates were identified based on Grams reaction and biochemical tests. The degradation efficiency was confirmed by GC-MS analysis, which indicated that the microbial isolates utilized most of the crude oil components. The result shows that the mean microbial counts for both TAHB decrease from 5.93 ± 0.1 to 5.38 ± 0.08 x10^{5}cfu/g during 56 days period of the study. The result also shows an increase in the mean counts of HUB from 0.00 ± 0.0x10^{3} to 3.74 ± 0.03x10^{3} cfu/g. Particle size A has the highest increase. The HUB identified were *Bacillus* spp., *Pseudomonas* spp., *Staphylococcus* spp., *Escherichia coli* and *Proteus* spp. The results indicate that larger particle size favors faster hydrocarbon biodegradation.
Keywords: Reclamation, Biodegradation, Hydrocarbon

INTRODUCTION

An increase in the world's population has led to an upsurge in the demand for petroleum and petroleum products, which has apparently become a source of pollution to the environment (Akoachere *et al.,* 2008). The wide spread contamination of most of arable lands, creeks, swamps and natural source of water with petroleum and petrochemical products particularly in the Niger Delta region of Nigeria, is due to an increasing petroleum exploration, refining and other related industrial activities (Okpokwasili and Odokuma, 1990; Odokuma and Okpokwasili, 1993). The contamination of these habitats poses major public health and socio-economic hazard which most often has developed into impetuous protestation between some of the oil companies and the surrounding communities (Akpe *et al.,* 2015).

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Soil texture and soil structure are both unique properties of the soil that have a profound effect on the behavior of soils, such as water holding capacity, nutrient retention and supply, drainage, and nutrient leaching (Silva *et al.,* 2000). The texture of soil is determined primarily by the size of the mineral particles that make up the soil (Oades, 1993). The mineral particles of a soil are present in a wide range of size and in association with organic matter (Tisdall, 1994). Fine earth fraction includes all soil particles that are less than 2 mm. Soil particles within this fraction are further divided into three (3) separate size classes, which includes sand, silt, and clay. The size of sand particles range between 2.0 and 0.05 mm; silt, 0.05 mm and 0.002 mm; and clay, less than 0.002 mm (Silva *et al.,* 2000).

Soil is a rich source of microorganisms capable of degrading hydrocarbons and residual oil (Atlas and Bartha, 1999). The rate of crude oil biodegradation in the soil seems to be rapid (Allamin, 2014). This may be due to the fact that the microorganisms in the soil have efficient ability in utilizing the residual crude oil as a source of carbon and energy. Indigenous and adapted microorganisms are more efficient for biodegradation of oil pollutant Akoachere *et al.* (2008).

Consequently, there is an increased interest in promoting environmental methods in the process of cleaning oil polluted sites. These methods are less expensive and do not introduce additional chemicals to the environment compared to the physicochemical methods (Akpe *et al.,* 2015).

Bioremediation using microorganisms capable of detoxify oil contaminated sites offers a very feasible alternative for an oil spill response due to the fact that majority of the molecule in the crude oil are biodegradable.

Many research activities have been conducted on bio-stimulation and bio-augmentation techniques of bioremediation, but not much has been done on natural attenuation. Therefore, this research which employed natural attenuation technique was aimed at studying the performance of biodegradation on different soil particle sizes which could entails decision when planning for hydrocarbon cleanup.

MATERIALS AND METHODS

Sampling Site and Sample Collection

Soil sample were randomly collected from different locations within the botanical garden of Bayero University Kano, using soil auger at a depth of 10cm (Onifade and Abubakar, 2007). The sample were transported to the microbiology laboratory in a black polythene bags which were mixed together and allowed to air dried.

Bonny light crude oil was collected from Kaduna Refinery and Petrochemical Company (KRPC), Kaduna, Nigeria.

Sieve Analysis of the Soil Sample

The air-dried soil samples were sieved into four (4) different particle sizes using sieve number; 0.6mm, 0.3mm, 0.15mm and 0.075mm respectively. The sieves were stacked with the largest opening at the top and smallest opening at the bottom. The soil was poured into the top of the sieve and covered. The set of sieves were put into a mechanical shaker for 15 minutes. When the shaker stopped, the stacks of sieves were removed. The retained soil samples on each sieve were poured into a sterile plastic container for further analysis (Sandra, 2011).

Biodegredation Experimental Setup

Exactly two hundred (200) grams each of the sieved soil particle sizes; (0.6mm, 0.3mm, 0.15mm and 0.075mm), were weighed and introduced into a masonjar bottles. Sixty mills (60ml) of crude oil was also added (to serve as the sole carbon and energy source), and allow to diffused through for each of the four different soil particle sizes. It was then shaken gently to ensure homogeneity. These setups were replicated in triplicated for each of the (4) different soil particle sizes, with each having two (2) different controls. These were incubated aerobically with perforated aluminum foil on top of the bottles at room temperature for fifty-six (56) days. Finally, a total of twenty (20) sets were generated as presented below;

A$_{= 200g}$ (0.6mm) soil particle size + 60ml crude oil (3×test)

B$_{= 2008}$ (0.3mm) soil particle size + 60ml crude oil (3×test)

C$_{= 200g}$ (0.15mm) soil particle size + 60ml crude oil (3×test)

D $_{== 200g}$ (0.75mm) soil particle size + 60ml crude oil (3×test)

1st CONTROL = 200g particle size + 60ml crude oil + Sterilization (1x for each particle size)

2nd CONTROL = 200g particle size + 60ml crude oil + 4% formaldehyde (1x for each particle size)

Microbial counts and pH of the samples were monitored four times at 14 days interval. Also, at 56days of the experiment, the residual crude oil was extracted from the soil using Soxhlet method. The level of microbial degredation of the crude oil was determined using Gas chromatography/mass spectrophometric analysis.

Soxhlet Extraction

Fifty grammme (50g) of the contaminated soil sample was weighed and wrapped in 11mm Whiteman filter paper. It was gently inserted into the timble.120mills of petroleum ether and 60 mills of diclromethane were placed into the distillation flask.

The temperature was adjusted to 60^{o}C and setup was allowed to run until the solvent through distillation process washes the soil completely. The extract was allowed to air dry for 24hours before taken for GC-MS analysis.

Enumeration, Isolation and Identification of Microorganisms

Enumeration of Total Aerobic Heterotrophic and Hydrocarbon Utilizing Bacteria

Enumeration of total aerobic heterotrophic bacteria (TAHB) was done on nutrient agar (NA) and Hydrocarbon Utilizing Bacteria (HUB) on Bush Knell Haas agar (BH) supplemented with crude oil. One (1) gram each of the soil samples was weighed and placed into test tubes containing 9ml of distilled water the test tubes were shaken vigorously in order to dislodge the microorganisms that adhered to the soil particles. The content of the tube were serially diluted from 10^{1} to 10^{4}. 10 µL of dilution factor 10^{-4} were plated in triplicates on to sterile Nutrient agar (NA) and (10^{-2}) on Bush knell Haas agar (BH) respectively. The plates were incubated at room temperature (30 ± 2^{0}C) for 48 hours and 7days for NA and BH respectively (Allamin, 2014). The colonies, which developed on the plates were counted and recorded as colony forming units per gram (cfu/g) of soil after Bashir (2012). Pure cultures of the isolates were obtained by repeated sub-culturing on media used for primary isolation. The pure isolates were maintained on agar slants for further characterization and identification (Abioye *et al.,* 2012; Allamin, 2014).

Morphological and Biochemical Identification

The phenotypic and biochemical characteristics used in identifying bacteria isolated included Gram staining, colonial appearance, motility, spore forming, urease, catalase, indole, oxidase citrate and methyl red Voges proskaeur. The tests were performed using the methods of Cheesbrough (2006).

Measurement of Rates of Crude Oil Biodegradation

Gas chromatographic/ Mass Spectrophotometric Analyses (GC-MS) of Oil Extracts

At fifty- six (56) days of the experiment, the residual crude oil were extracted from the soil using Soxhlet method. The level of microbial degradation of the crude oil was determined using Gas chromatography/ mass spectrophometric analysis as described by Ijah (1998); Ibrahim *et al.* (2009).

Determination of Physiocochemical Properties of the Soil Sample

Total carbon was determined by dichromate wet oxidation method of Walkey and Black as modified by Dyan *et al,* (1999). Nitrogen content was determined using the micro kjeldah digestion method of Brady and Weil (1999). Particle size was determined using method described by Sheldrick and Want (1993). Moisture content was determined using the method of Morris (1999).

Statistical Analysis of Data

The data in this study were subjected to one-way analysis of variance (ANOVA) using instant GraphPad software version 3.05. Relation between variables and comparison of means were tested for level of significance at $p \leq 0.05$.

RESULTS AND DISCUSSION

The soil sample used for the study was composed of 72% sand, 22% silt and 7% clay. The textural class of the soil was sandy, clay and loam. The total nitrogen content of the soil sample was 0.18%, the organic carbon content was 1.5% while the phosphorus content 32.53 mg/kg. The moisture content was 1.02% (Table 1). The presence of these limiting nutrients (C, N and P) in the soil samples analyzed in this study is in consonance with previous reports (Okoh, 2006).

The low level of nutrients in the soil sample could have been caused by leaching or erosion.

This agrees with the findings of Romanus *et al.* (2015) who made similar observation of low percentage of carbon, nitrogen and phosphorus content of a garden soil which was used for bioremediation studies. Soils with appreciable content of these elements have been implicated in bioremediation studies.

The pH of the soil sample monitored during the period of study ranged from 6.55 to 7.66 as shown in (Table 2). The result of the pH observed in this study is within the favorable range for biodegradation of crude oil in polluted soil. Similar observations have been reported by Romanus *et al.* (2013) and Akpe *et al.* (2015).

Table 1: Physicochemical Parameters of the Soil Sample

Sample	Physicochemical Parameters of the soil sample								
	pH	P (mg/kg)	O.C %	N%	MC%	% Sand	% Silt	% CAY	TC
A	7.01	32.53	1.58	0.18	1.02	72.00	22.00	7.00	Sandy, Clay, Loam

Key; PH, O.C=ORGANIC CARBON, N= NITROGEN, MC=MOISTURE CONTENT, TC=TEXTURAL CLASS

Table 2: pH of Crude Oil Polluted Soil Having varying degrees of Particle Size.

Time(days)	Sample A	Sample B	Sample C	Sample D
0	6.50	6.53	6.74	6.79
14	6.73	6.54	6.63	6.50
28	7.20	7.52	7.66	7.64
42	6.84	6.85	6.93	6.81
56	6.84	6.85	6.93	6.81

KEY: A=0.66mm particle size B=0.3mm particle size C =0.15mm particle size D= 0.75mm particle size

The results of the Total aerobic heterotrophic bacteria (TAHB) indicated that the mean microbial counts of TAHB reduced from zero to 56 days of the study (Table.3). The highest reduction was observed to be in soil with the largest particle size (0.6mm) which ranged from $5.93 \pm 0.01 \times 10^5$cfu/g to $5.5 \pm 0.06 \times 10^5$ cfu/g. This could be due to lower water retention capacity of the soil particle size which greatly enhanced hydrocarbon loss. This is in agreement with findings by Sandra (2011), who reported higher loss of hydrocarbons in soil with larger grain size. It also agreed with the study of Kogbara *et al.* (2015) who observed faster rate of hydrocarbon loss in sandy soils. The result also indicated that there were no wide difference in the reduction of mean microbial counts among particle sizes B, C and D. This could be due to them having higher surface area which impede on the ability to efficiently degrade hydrocarbons. This is in line with the work reported by Cui *et al.* (2011); Sandra (2015) and Romanus *et al.* (2015).

The decrease in the heterotrophic microbial counts could be as a result of adaptation to the polluted environment as well as the toxic effect of crude oil on the microbial population as previously suggested Akoachere *et al.* (2008) and Mbah *et al.* (2009).

Generally, the total heterotrophic bacterial counts were found to be higher than the crude oil utilizing bacterial in all the samples. This could be as a result of nutrient limitation in the enumeration media for crude oil utilizes. This finding is consistent with work Romanus *et al.* (2015), who equally reported higher heterotrophic bacteria counts than hydrocarbon carbon counts in crude oil polluted soils.

Table 3: Total Aerobic Heterotrophic Bacteria Count of the Soil Sample (×10^5 cfu/g)

Samples	Day 0	Day 14	Day 28	Day 42	Day 52
A	5.93±0.01^{*a}	5.86±0.02**	5.62±0.04*	5.87±0.05*	5.55±0.06*
B	5.83±0.03^{*bc}	5.78±0.02*^{a}	5.65±0.05*	5.40±0.06*	5.51±0.05*
C	5.85±0.06*^{ab}	5.72±0.04*^{a}	5.60±0.05*	5.53±0.04*	5.42±0.05*
D	5.75±0.04^{*c}	5.62±0.04*	5.60±0.06*	5.51±0.03*	5.38±0.08*
E	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
F	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

KEY; A=0.6mm, B=0.3mm,C=0.15mm,D=0.075mm, E = Control, F = Control, Cfu/g=colony forming units per gram, values were Mean ± SD of triplicates determinations. Values with single * = not significantly different. ** = significantly different, the same alphabets combination along the rows were not significantly different ($p<0.05$).

Table 4: Hydrocarbon Utilizing Bacterial Counts of the Soil Sample (×10^3 cfu/g)

Samples	Day 0	Day 14	Day 28	Day 42	Day 56
A	0.00±0.00*	3.23±0.06**	3.42±0.09*	3.65±0.07*^{a}	3.74±0.03**
B	0.00±0.00*	3.01±0.02*^{ab}	3.21±0.07*	3.56±0.04^{abc}	3.57±0.03*^{ab}
C	0.00±0.00*	3.03±0.05*^{ac}	3.18±0.11*	3.58±0.02*^{bd}	3.56±0.02*^{ac}
D	0.00±0.00*	2.87±0.11*^{bc}	3.18±0.11*	3.49±0.02*^{cd}	3.57±0.01*^{bc}
E	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
F	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

KEY; A=0.6mm,B=0.3mm,C=0.15mm,D=0.075mm, E = Control, F = Control, Cfu/g=colony forming units per gram, values were Mean ± SD of triplicates determinations. Values with single * = not significantly different. ** = significantly different, the same alphabets combination along the rows were not significantly different ($p<0.05$).

Results of the hydrocarbon utilizing bacteria (HUB) counts indicated that there were no microbial activities at zero day of the study (Table 4). This may suggest period of adjustment and adaptation to the new environment by the indigenous microorganisms. It was observed that HUB counts generally increased from 14 to 56 days of the study. This could have resulted from the adjustment of the indigenous microorganisms to the new environment and their ability to synthesize other metabolites suspected to have been generated during the biodegradation period and conforms with the findings by Akoachere *et al.* (2008). The larger particle size 0.6 mm (A) has the highest HUB counts. This could have resulted from the lower surface area which provided better aeration that greatly enhanced the ability of the microbes to attack hydrocarbons and utilized them as sole carbon and energy source for their metabolic activities. This is consistent with the study of Kogbara *et al.,* (2015) who reported that sand with larger particle sizes favors faster hydrocarbon degradation due to enhance aeration and thus improved microbial survival and activity during crude oil contamination as well as in the course of remediation. This result also agreed with the existing literature (Romanus *et al.,* 2015; Sandra, 2015).

Analysis of variance for HUB showed significant difference ($P \leq 0.05$) between particle sizes A, B, C and D. However, there were no significant difference among B, C and D. This could be due to their higher specific surface area.

Table 5: Morphological and Biochemical Characteristics of Hydrocarbon Utilizing Bacteria Isolated from the soil sample

Isolate	Biochemical Characteristics																Bacteria
	GR	Shape	Spore	CAT	OXI	MR	VP	UR	IND	CIT	TSI GLU	TSI LAC	TSI SUC	TSI H_2S	TSI GAS	TSI MOT	
1	-	Rods	-	+	-	+	-	+	+	-	+	-	-	+	+	+	*Proteus vulgaris*
2	+	Rods	+	+	-	-	+	+	+	+	+	-	-	-	-	+	*Bacillus cereus*
3	-	Rods	-	+	+	+	-	-	-	+	+	-	-	-	-	+	*Pseudomonas putida*
4	-	Rods	-	+	-	+	-	-	+	-	+	+	+	-	+	+	*Escherichia coli*
5	+	Cocci	-	+	-	-	-	-	-	-		-	-	-	-	-	*Staphylococcus aureus*

Keys: + =positive, - =negative, CAT= Catalase, OXI= Oxidase, MR= Methyl-red, VR=Voges Proskauer, UR= Urease, IND = Indole, CIT= Citrate, GLU=Glucose, LAC=Lactose, SUC=Sucrose, and MOT=Motility, TSI=Triple sugar ion test.

The bacteria isolated from this study were *Bacillus cereus, Proteus vulgaris, Pseudomonas putida, Escherichia coli* and *Staphylococcus aureus* (Table 5). The predominant form were rod shape bacteria and *cocci*. The Gram-negative bacteria were mostly isolated. These and other bacterial species isolated from the soil sample are shown in Table 7. The result also correlates the report of previous workers (Esumeh *et al.,* 2009) who isolated more of Gram negative organisms suggesting that they are better degraders of crude oil when compared with their Gram positive counterparts. The higher ability of Gram-negative bacteria to utilize crude may not be unconnected with the possession of plasmid-borne or chromosomal genes involved in hydrocarbon degradation and porins in their cell wall which helps in the uptake of certain substances by the cell or extrusion of others which may be harmful (Vahaboglon *at al.,* 1996; Jørgensen *et al.,* 2000; Akpe *et al.,* 2013). The crude utilizing bacteria identified in this study have been identified and implicated in crude oil biodegradation by several investigators (Antai, 1990; Allamin 2014; Romanus *et al.,* 2015; Ajayi *et al.* 2008).

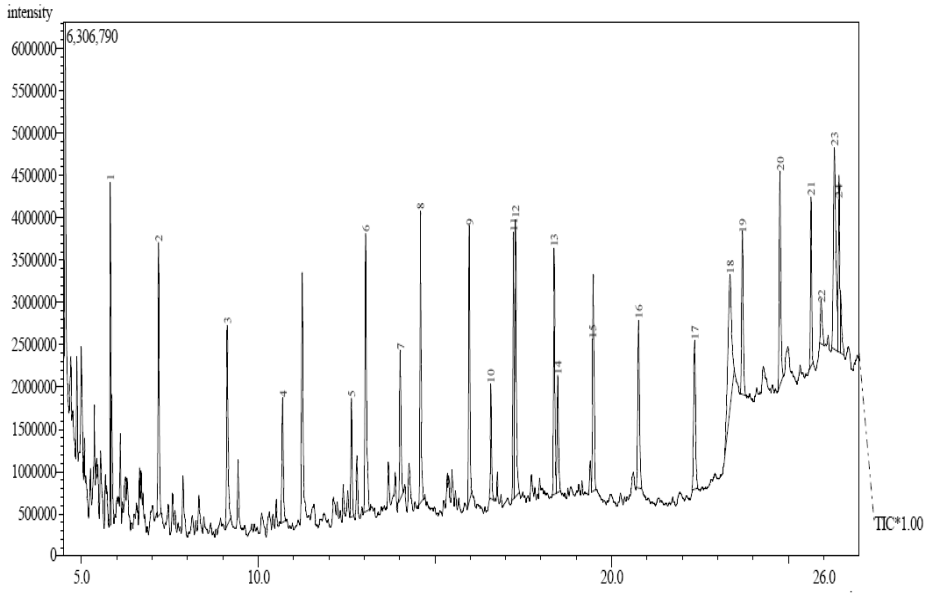

Figure 1: Gas Chromatogram of the Raw Bonny Light Crude Oil (Negative Control).

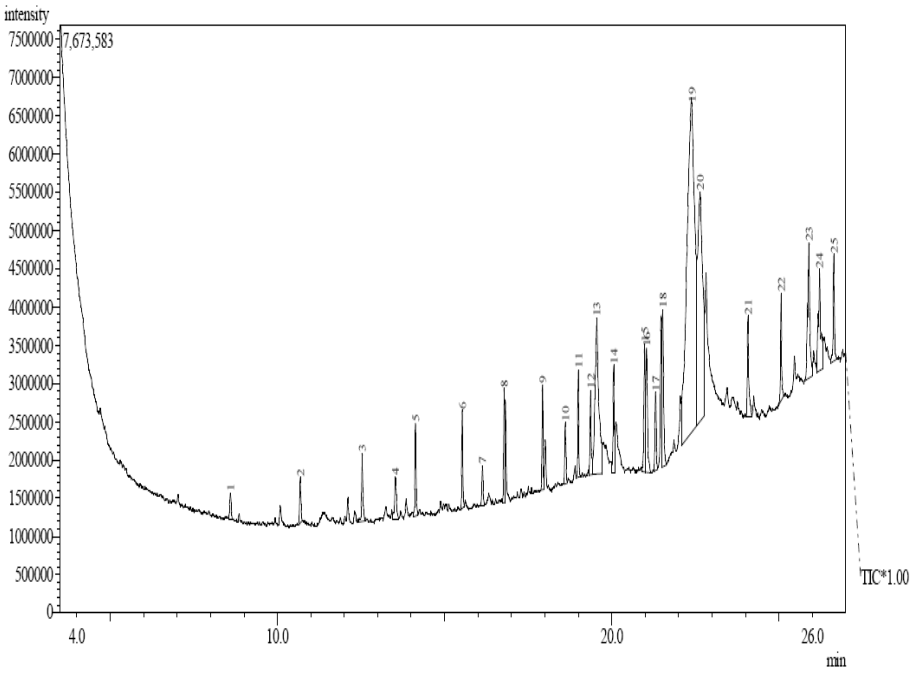

Figure 2: Chromatogram of Soil Particle Size A (0.6mm) Degraded by Indigenous Bacteria after 56 Days of Incubation.

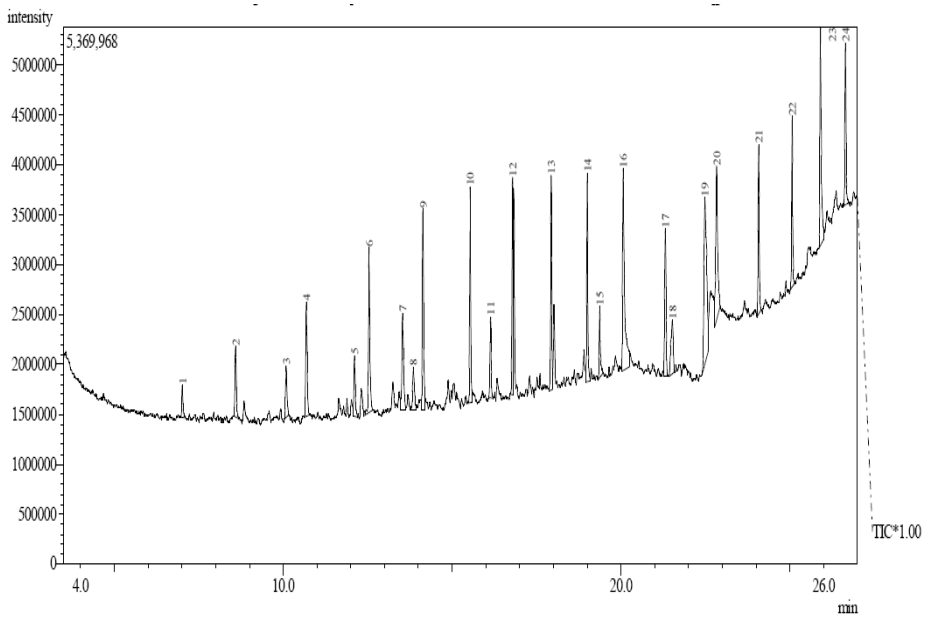

Figure 3: Chromatogram of Soil Particle Size B (0.3mm) Degraded by Indigenous Bacteria after 56 Days of Incubation

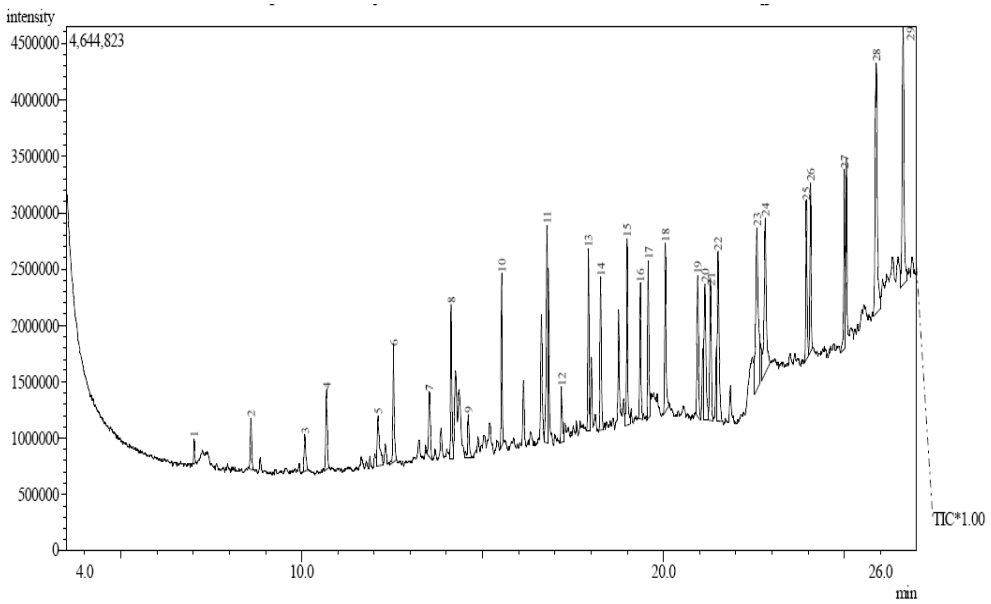

Figure 4: Chromatogram of Soil Particle Size C (0.15mm) Degraded by Indigenous Bacteria after 56 Days of Incubation

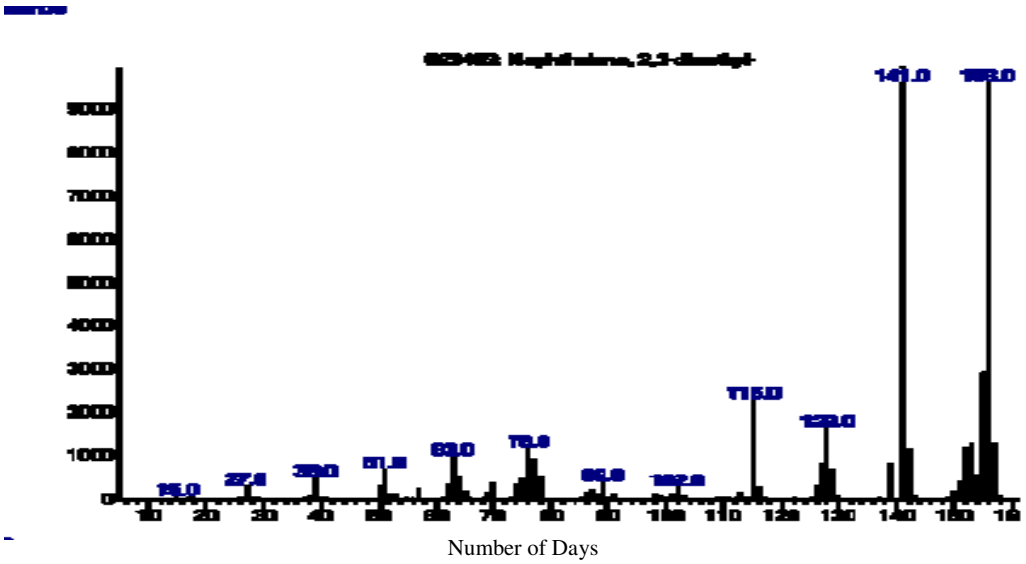

Figure 5: Chromatogram of Soil Particle size D (0.75mm) Degraded by Indigenous Bacteria after 56 Days of Incubation

The result of the GCMS analysis (Figures 1 - 5) further confirmed the biodegradation potentials of the isolates. GCMS analysis was conducted at 14, 28 and 56 days of the study. It was observed that the highest biodegradation occurs at 56 day. This revealed that biodegradation through natural attenuation takes longer time. This finding is consistent with previous work of Victor *et al.,* 2015, who made similar observation that natural attenuation occurs in his control sample (1kg soil 100ml spent engine oil) at 70 days of the study due to 50% removal of the oil. The result further shows that some components of the oil, particularly the lighter hydrocarbons were completely lost while lower molecular weight were reduced in peak height, indicating that degradation of the component has occurred. Other components of the oil were intact.

This is in agreement with the results of Kawo and Faggo (2017) and Maryam and Ijah (2015) who also reported a decrease in peak height of chromatogram as indication of degradation. It was observed that highest degradation occurred on particle A, followed by B, C and D (Figures 2-5). Generally, the accepted pattern of susceptibility of hydrocarbon components to microbial degradation is n-alkane > branched alkanes > low-molecular weight aromatics > polycyclics. However, system-specific exceptions to this pattern have been found (Makut and Ishaya, 2010).

The findings further suggest that among the four soil particle sizes encountered in this study, the relative ease of oil degredation via natural attenuation is in the order 0.6mm>0.3mm>0.15mm>0.075mm. This study provides information on how different soil particle sizes interact with hydrocarbons that would aid in oil spill bioremediation.

CONCLUSION AND RECOMMENDATION

The study concluded that larger particle size (0.6mm) favors faster hydrocarbon biodegradation. The study also revealed the presence of *Pseudomonas putida, Staphylococcus aureus, Proteus vulgaris, Bacillus cereus,* and *E. coli.* The study also concluded that biodegradation can be influenced by soil particle sizes, concentrations and types of the organisms present in the systems.

REFERENCES

Abioye, O. P., Agamuthu, P. and Abdul Aziz, A. R. (2012). Biodegradation of used lubricating oil by microbes' isolated from pristine soil environment *Malaysian Journal of Science* **31** (1): 1-7.d.

Ajayi, A. O., Balogun, S. A. and Adegbehingbe, K. (2008). Microorganisms in the crude oil-producing areas of Ondo State. Nigeria. *Academic Journals Scientific Research and Essay* 3 (5): 174-179.

Akoachere, J.T.K., Akenji, T. N., Yongabi, F.N., Nkwelang, G. and Ndip, R. N. (2008). Lubricating oil degrading bacteria in soils from filling stations and automachanic workshops in Buea, Cameroon: occurrence and characteristics of isolates. *African. J. Biotechnol.* 7, 1700-1706.

Akpe, A. R.**,** Ekundayo, A. O. and Esumeh, F. I. (2013). Degradation of Crude oil by bacteria: A role for plasmid-borne genes. *Global Journal of Scientific Frontier Research Biological Science 13*(Issue 6 Version 1.0): 21-26. Code 279999p

Akpe, A. R**.,** Esumeh, F. I., Aigere, S. P., Umanu, G. and Obiazi, H. (2015). Efficiency of plantain peels and guinea corn shaft for bioremediation of crude oil polluted soil. *Journal of Microbiology Research 5* (1): 31-40. DOI: 10.5923/j. microbiology. 20150501.04

Allamin, I. A. (2014). Occurrence of hydrocarbon degrading microorganisms in soil in Kukawa,Borno State, Nigeria. A dissertation submitted to the school of post graduate, Usman Danfodiyo University Sokoto, Nigeria.

Antai, S. P. (1990). Biodegradation of Bonny light crude oil by *Bacillus* spp. and *Pseudomonas* spp. *Waste Management* 10: 61-64.

Atlas, R. M. and Bartha, R. (1999). *Microbial Ecology: Fundamentals and Applications* Benjamin/Cumming Publishing Company, California, pp.17-435.

Atlas, R.M. (1981) Microbial degradation of petroleum hydrocarbons: an environmental perspective. *Microbiological Reviews* (45): 180-209.

Bashir, A. H. (2012). Diversity and morphology of bacterial community characterized in topsoil samples from the Gaza Strip, Palestine. *Research Journal of Microbiology* 7(6): 309-318.

Brady, N. C. and Weil, R. R. (1999). *The Nature and Properties of Soils.* 12th ed., Prentice Hall Publishers London. Pp 740.

Chessbrough, M. (2006). *District laboratory practice in tropical countries*; part 2. Second edition, Cambridge University press, UK, PP. 33-70.

Dyan, S, Chhonkar, P.K, and Pandey, R.N. (1999). Soil plant and water analysis a method manual IARI, Delhi.

Cui, X, Hunter, W, Yang, Y, Chen, Y., and Gan J. (2002) Biodegradation of pyrene in sand, silt and clay fractions of sediment. Biodegradation, 2011; 22(2): 297-307

Esumeh, F. I., Akpe, A. R., and Eguagie, O. E. (2009). Crude oil Degrading Capabilities of bacterial isolates from pawpaw (*Carica papaya*) and sweet orange (*Citrus sinensis*). A role for plasmid mediated gene.

Foght, J. M. and Westlake, D. W. S. (1987). Biodegradation of hydrocarbon in fresh waters, In: Vandermuelen, J. H. Hrudy, S. E. (eds.) *Oil in Freshwater: Chemistry, Biology, Countermeasure Technology.* Pergamon Press, New York. Pp. 252-263.

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

Jørgensen, K. S., Puustinen, J., and Suortti, A. M. (2000). Bioremediation of petroleum hydrocarbon-contaminated soil by composting in biopiles. *Environmental Pollution*,107 (2): 245–254.

Jyothi, K., Surendra, B. K., Nancy, C. K. and Kashyap, A. (2012). Identification and isolation of hydrocarbon degrading bacteria by molecular characterization. *Helix* 2:105-111

Kawo, A. H. and Fago, A. A. (2017). Ethanol removal of crude on in soil b culture of *Bacillus subtilis* and *Pseudomonas aeruginosa* isolated from contaminated soil in Kano State, Nigeria. *Bayero Journal of Pure and Applied Sciences* (BAJOPAS), 423 – 427.

Kayode-Isola, T. M., Eniola, K. I. T., Olayemi, A. B. and Igunnugbemi, O. O. (2008). Response of a resident bacteria of a crude oil polluted river by diesel oil. *American-Eurasian Journal of Agronomy* 1(1): 6 – 9.

Kogbara, R. B., Ayotamuno, M. J., Worlu, D. C., and Manuel, I. F., (2015). A case study of petroleum Degradation in different soil textural classes.

Maryam L. R. S. and Udeme J. J. Ijah (2016). "Ethanol removal of crude oil in soil by mixed culture of *Bacillus megaterium* UL05 and *Pseudomonas aeruginosa* UL07" *International Journal of Environmental Bioremediation and Biodegradation* Vol. 4 No. 1 8 – 12.

Makut, M. D. and Ishaya, P. (2010). Bacterial species associated with soils contaminated with used petroleum products in Keffi town, Nigeria. *African Journal of Microbiology Research* 4(16): 1698-1702.

Mbah, C. N., Nwite, J. N. and Nweke, I. A. (2009). Ameriolation of spent oil contaminated ultisol with organic wastes and its effect on soil properties and maize (*Zea mays L*) yield. *World. J. Agric. Sci***.** 5(2), 163-168

Moneke, C. and Nwangwu, A. (2011). Studies on the bioutilization of some petroleum hydrocarbons by single and mixed cultures of some bacterial species. *African Journal of Microbiology Research* 5 (12): 1457-1466.

Oades, J.M. (1993). The role of biology in the formation, stabilization and degradation of soil structure Geoderma, 56; 377-400.

Odokuma, L. O., and Okpokwasili, G. C. (1993). Seasonal ecology of hydrocarbon-utilizing microbes in the surface waters of a river. *Environ Monit Assess.,* 27: 175–191.

Ofoegbu, R. U., Momoh Y. O. L., and Nwaogazie I. L. (2015) Bioremediation of crude oil contaminated soil using organic and inorganic fertilizers. *Journal of Petroleum and Environmental Biotechnology*. 5: 198.

Okpokwasili, G. C., and Odokuma, L. O. (1990) Effect of salinity on biodegradation of oil spill dispersants. *Waste Manag.,* 10: 141–146.

Okoh, A. I. (2006). Biodegradation alternative in the cleanup of petroleum hydrocarbon pollutants *Biotechnology and Molecular Biology Review* 1(2) 38-50.

Onifade, O. A. and Abubakar, F. A. (2007). Characterization of hydrocarbon-degrading microorganisms from isolated from crude oil contaminated soil and remediation of the soil by enhanced natural attenuation. *Research Journal of Microbiology* **2**(2): 149-155.sandy soils. M Eng. Thesis; University of Nigeria.

Romanus, A. A., Ekundayo, A. O., Aigere, S. P. and Okwu, G. I. (2015). Bacterial Degradation of Petroleum Hydrocarbons in crude oil polluted soil amended with cassava peels. *American journal of Research Communication,* 3(7); 99-118

Sandra, A. C. S. (2011). Effects of particle sizes bioremediation of crude on polluted Sandy soils. A Masters thesis submitted to the Department of Civil Engineering, faculty of Engineering, University of Nigeria NsukkaM Eng.Thesis; University of Nigeria, Nsukka.

Silva, J. and Uchida, R. S. (2000). *Plant Nutrient Management in Hawaii's Soils: Approaches for Tropical and Subtropical Agriculture.* College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa, Honolulu.

Sheldrick, B.A. and Want, C. (1993). Particles size distribution soil sampling and methods of analysis, Canadian society of soil science, lewsi publishers: 499 – 511.

Tisdall, J. M. (1994). Possible role of soil microorganisms in aggregation in soils. Plant and soil, 159;115-121. United Nations Development Programme, Niger Delta Human Development Report. Abuja, Nigeria, 2006:185and186

Vahaboglon, H., Dodanli, S. and Ozturk, R. (1996). Characterization of multiple antibiotic resistant *Salmonella typhimurium* strains, molecular epidemiology of PCR-1-producing isolates and evidence for nosocomial plasmid exchange by a clone. *J. Clin Microbiol. 34*: 2942 -2946

Victor Taghoghor Omoni, Celestine Uzoma Aguoru , Emmanuel Odogbo Edoh and Oluwatayo Makinde (2015) Biostimulation of hydrocarbon utilizing bacteria in soil contaminated with spent engine oil using banana and plantain agro-wastes. *Journal of Soil Science and Environmental Management* Vol. 6(8), pp. 225-233.