ImpactImpacts (since it isn't just one impact) of Artisanal Crude oil Refining Activities on Soil Microorganisms

Abstract

Illegal crude oil refining activities have devastating consequences on the soil environment where most of these activities take place.

Aim: To evaluate the effect of illegal crude oil refining activities on soil microorganisms using standard microbiological methods.

Study design: This study employs laboratory experimental design, statistical analysis of the data and interpretation.

Place and Duration of Study: Soil samples were taken once a month for three months (May-July, 2018) from Ke in Degema Local Government Area of Rivers State, Nigeria, where illegal crude oil refining activities are ongoing.

Methodology: Using standard microbiological methods, total culturable heterotrophic bacterial counts, total fungal counts, hydrocarbon utilizing bacterial and fungal counts were analysed to evaluate the effect of the activities. Total hydrocarbon content of the soil samples was also analysed.

Results: The populations of the total heterotrophic bacterial, fungal and hydrocarbon utilizing bacterial (HUB) should be here since it's the first mention and fungal (HUF) counts of the contaminated soil were enumerated. The mean total heterotrophic bacterial counts in Station 1 around the pot ranged from 2.5 x 10^5 to 1.8 x 10^6cfu/g, fungal counts ranged from 2.1 x 10^3 to 4.4 x 10^4cfu/g, hydrocarbon utilizing bacterial (just HUB will be sufficient here and henceforth) (HUB) counts ranged from 4.2 x 10^4 to 6.4 x 10^5cfu/g and hydrocarbon utilizing fungal (HUF) counts ranged from 1.5 x 10^3 to 4.0 x 10^3cfu/g. The results of soil samples taken 20m away from the Pot location ranged from 7.0 x 10^5 to 8.2 x 10^6cfu/g for total heterotrophic bacterial counts, fungal counts ranged from 2.3 x 10^3 to 1.5 x 10^4cfu/g, HUB ranged from 4.7 x 10^4 to 5.7 x 10^5cfu/g and HUF ranged from 2.0 x 10^3 to 3.5 x 10^3cfu/g. Also, the results of total heterotrophic bacterial counts for Station 2 ranged from; 4.3 x 10^5 to 3.3 x 10^6cfu/g, fungi 2.0 x 10^3 to 3.3 x 10^4cfu/g, HUB ranged from 3.8 X 10^4 to 5.4 x 10^4cfu/g and HUF 1.6 x 10^3 to 3.5 x 10^3cfu/g, while 20m away from the Pot total heterotrophic bacteria ranged from 1.3 x 10^7 to 6.5 x 10^7cfu/g, fungi 5.8 x 10^3 to 1.4 x 10^5cfu/g, HUB 5.4 x 10^4 to 1.1x 10^5cfu/g and HUF 3.1 x 10^3 to 4.7 x 10^4cfu/g. While the control samples taken from inside the community where no such activity is on, ranged from 2.6 x 10^7 to 7.9 x 10^7cfu/g for total heterotrophic bacteriabacterial counts, total heterotrophic fungal counts ranged from 2.8 x 10^4 to 5.3 x 10^4cfu/g, HUB 2.0 x 10^2 to 3.1 x 10^2cfu/g and HUF 2.0 x 10^1 to 2.3 x 10^1cfu/g. twelve bacterial genera were identified and eight fungal genera: *Bacillus, Alcaligenes, Flavobacterium, Acinetobacter, Pseudomonas, Micrococcus, Proteus, Serratia, Enterobacter, Streptococcus, Escherichia*, *Staphylococcus*, *Penicillum, Aspergillus, Fusarium, Mucor, Rhizopus, Geotrichum, Candida*, and *Cladosporium*.

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Total hydrocarbon content range from 106 to 281mg/kg across the locations. When compared with the control, it was observed that the microbial population and diversity were adversely affected. These variations observed in the microbial population are indicative of the effect of the illegal refinery on the soil microorganisms.

Conclusion: The results of this study indicates that the continuous contamination of the soil environment by the activities of illegal crude oil refining, lead to a decrease in microbial population and diversity. This may result in devastating ecological damage, adversely affecting the ecological balance which may affect food chain and in turn animals and humans.

Keywords: illegal crude oil refining, soil bacteria, fungi, population, diversity

Introduction

The discovery and large scale production of crude oil in the Niger Delta region have exposed this region to great crude oil pollution challenge. This region in the past years have experienced the devastating effect of oil spills into both the terrestrial and aquatic environments(Chikere and Ekwuabu, 2014).This results from oil refining operations, transport, equipment failure, accident, bunkering activities and also illegal crude oil refining activities (Douglas, 2018). Research has shown that, between 200,000 – 300,000 barrels of oil are lost daily due to oil thefts out of which about 75% is sold offshore while the remaining 25% are refined locally (Obenade and Amangabara, 2014; Douglas, 2018). The soil ecosystem is directly affected since, most of these activities take place here, resulting in the discharge of crude oil and its products at various levels of refining and waste products released. These components greatly impact on plants, animals and microorganisms that depend on the nutrients in the soil for their survival. It reduces plant growth, affects aeration by blocking soil pores, thereby creating anaerobic conditions (Njoku *et al*., 2016). When crude oil is refined, various hydrocarbon fractions are produced, which have obvious eco-toxicological impacts on the environment when spilled. These impacts include; reduction in biodiversity, changes in soil physicochemical characteristics, groundwater contamination, adverse effect on microflora, bioaccumulation and biomagnifications in environmental receptors, alteration of the habitat and cancer in humans (Obire and Anyanwu, 2009; Kalantary *et al*., 2014). Toxicity of these products varies, which depends on the concentration, composition, the prevailing environmental conditions and the biological state of the organism when the pollution occurs (Obire and Anyanwu, 2009).

Microorganisms play key role as indicators of the Health of aquatic and terrestrial ecosystems. This is due to their availability, abundance, their rapid growth, and ease of testing, which have made them an important tool in pollution monitoring. Microorganisms are very sensitive to changes or fluctuations in their environment, which is why they are used as microbial indicators of pollution (Parmar *et al*., 2016). The increased input of crude oil and petroleum products into the environment have produced an enriched microbial community, which is able to survive in such contamination (Chikere *et al*., 2009). Whenever, there is a sudden alteration in their physical or chemical environment, it results in a period of lag phase in which the microbial

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population adapts to the new conditions. This lag phase is also called acclimatization phase, which enables the organisms to acquire metabolic abilities for survival in the environment (Chikere *et al*., 2009). Microorganisms have the ability to respond to low levels of pollutants and other biological and physicochemical changes in the environment (Parmar *et al*., 2016). The microbial communities in the soil ecosystem are responsible for food chain/web, nutrient recycling and biodegradation.

Research has revealed that bacteria have the highest population in the soil, and they are most adapted to use hydrocarbon as a source of carbon and energy. Whenever, crude oil and petroleum products are spilled into the soil ecosystem, the microbial community structure is altered and diversity reduces due to environmental stress or alteration which results in the production of dominant populations within the altered communities which can withstand such contamination with improved substrate utilization and physiological abilities (Atlas and Philip, 2005; Kumar and Khanna, 2010). This research was carried out to evaluate the impact of the illegal crude oil refining activities on soil microorganisms. The keKe axis of the Degema Local Government Area of Rivers State, Nigeria houses several illegal crude oil refining sites and also a market for the refined products and other oil businesses.

Materials and Methods

Study Area

This study was conducted in two illegal crude oil refinery sites (designated as Station 1 and 2) in Ke, Degema Local Government Area, of Rivers State, Nigeria. The GPS Coordinates for Station 1 is Location 04^0 45' 33.6'' N, 007^0 00' 01. 0''E, and Station 2 is 04^0 45' 33.6'' N, 007^0 00' 01. 0''E.

3.2. Scope of Study

This study was carried out between May and July, 2018. Soil samples were collected at about 0-15cm depth using a soil auger into sterile bags, from four different points around the Pots. Pot here refers to the fabricated aluminum tanks used in the distillation process. For Station 1 it is designed as Pot 1 and soil samples bulked for homogeneity. Then, 20m away from the pot a second set of soil samples were also taken. Same was done for Station 2, soil samples were taken around the pot and 20m away from the pot (Pot 2). Control soil samples were taken inside the community, away from the illegal refining sites. These samples were labeled properly and immediately transported to the laboratory for analyses.

Enumeration of Total Heterotrophic Bacteria

The spread plate method was used to determine the total heterotrophic bacterial counts on nutrient agar. One gram of soil was taken from each soil sample and homogenized in 9mls of physiological saline. An aliquot of 0.1ml of the dilutions of 10^{-4} and 10^{-5} were plated out on the

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113 surface of the agar and evenly spread using a sterile hockey stick. Plates were incubated at 30^0C
114 for 24 hours. The colonies that developed on the plates were counted and mean calculated for
115 duplicate plates, results expressed in colony forming unit per gram (CFU/g)(Douglas and Green,
116 2015).

117 **Enumeration of the Hydrocarbon Utilizing Bacterial Population**
118 Hydrocarbon utilizing bacterial populations in the soil samples were enumerated using mineral
119 salt agar **(The components of the mineral salt agar and weights in gram should be listed
120 here).** The vapour phase transfer method using Mineral salt medium composition of Mills *et al*.,
121 1978 was used as modified by Okpokwasili and Okorie (1988). Aliquot (0.1ml) of the 10^{-4} to 10^{-5}
122 dilutions, previously obtained during the serial dilution of the soil samples, were inoculated in
123 duplicates on appropriately labeled mineral salt agar plates which was freshly prepared and
124 dried. The vapour phase transfer method in which a sterile Whatman No. 1 filter paper, placed on
125 the lid of the Petri plate is saturated with 5ml Bonny light crude oil. Plates were inverted and
126 incubated for 7days at 30^oC. Colonies were counted after incubation, average counts calculated
127 for duplicate plates and expressed as colony forming unit (CFU/g).
128 **Enumeration of Total Heterotrophic Fungi**
129 Spread plate method was used on Sabouraud Dextrose agar (SDA). An aliquot, 0.1ml of 10^{-3} and
130 10^{-4} dilutions were inoculated onto the freshly prepared SDA plates, in which 0.5% Ampicillin
131 has been added. This was done to inhibit bacterial growth while allowing the growth of fungi
132 (Cheesbrough, 2000). The inoculum was spread evenly using sterile hockey stick. Plates were
133 inverted and incubated at 28^0C for 5days. Colonies that developed on the plates were counted,
134 average counts on duplicate plates calculated and recorded as cfu/g.
135

136 **Enumeration of the Hydrocarbon Utilizing Fungi**
137 The MSA as composed by Mills *et al*., 1978 as modified by Okpokwasili and Okorie, (1988) to
138 which 5% tetracycline was added to prevent bacterial growth was used. This medium was used
139 for the isolation and enumeration of hydrocarbon utilizing fungi. From dilutions of 10^{-3} and 10^{-4},
140 0.1ml aliquot was transferred on the freshly prepared plates; evenly spread using the hockey
141 stick. The vapour phase transfer method in which a sterile Whatman No. 1 filter paper, placed on
142 the lid of the Petri plate is saturated with 5ml Bonny light crude oil. Plates were inverted and
143 incubated for 7days at 30^oC. Colonies that developed on the plates after incubation were counted,
144 average counts calculated for duplicate plates and expressed as colony forming unit (CFU/g) of
145 soil.
146

147 **Purification and characterization of Organisms**
148 Discreet colonies that developed on the Nutrient and Mineral Salt agar plates were subcultured
149 by streaking on Nutrient agar until pure cultures were obtained. Colonies on SDA and MSA for
150 fungi were also subcultured by streaking on SDA until pure cultures were obtained. Pure cultures
151 of bacterial and fungal isolates were preserved in bijou bottles containing nutrient and SDA
152 slants respectively. The pure isolates were then refrigerated and were used for other analyses.

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153 The pure bacterial isolates were further investigated by carrying out routine microbiological
154 analyses including; cultural and biochemical characteristics. The following test were done; Gram
155 staining, cell motility, oxidase, indole and catalase production, citrate utilization, methyl Red-
156 Voges Proskaeur test, acid/gas production from sugar fermentation, as described by Bergey's
157 Manual for Determinative Bacteriology (Holt *et al*., 1994).

158 **Identification of Fungal Isolates**

159 The fungal isolates were identified basically by both macroscopic and microscopic examination.
160 Macroscopic identification was done by observing the morphology of the pure cultures in the
161 plates. The microscopy was done by removing a small portion and placing on clean grease free
162 slide. Lactophenol blue was dropped on the slide and smeared, it was covered using a cover slip
163 and viewed under x10 and x40 objective lens (Cheesebrough, 2000). The observed
164 characteristics were recorded and compared with the identification key in Barnett and Hunter,
165 (1972).

166 **Determination of Total Hydrocarbon Content (THC)**

167 Total Hydrocarbon Content (THC) analyses were carried out on all soil samples using
168 spectrophotometric method. The total hydrocarbon content of the soil samples were determined
169 by shaking 10g of a representative soil sample with 20ml xylene and the oil extracted determined
170 by measuring the absorbance using a spectrophotometer at 420nm using a spectronic 20. A
171 standard curve of the absorbance of different concentrations of hydrocarbon concentration in the
172 soil sample was measured in g/g after reference to a standard curve and multiplying by the
173 appropriate multiplication factor (Nrior *et al*., 2017).

174

175 **Statistical Analysis**

176 Statistical analysis was performed using analysis of variance (ANOVA) using a computer based
177 SSPS version 22.

178 **Results**

179 The effect of illegal crude oil refining activities on soil microorganisms was investigated. The
180 mean total heterotrophic bacterial counts in Station 1 around the pot ranged from 2.5 x 10^5 to 1.8
181 x 10^6cfu/g, fungal counts ranged from 2.1 x 10^3 to 4.4 x 10^4cfu/g, hydrocarbon utilizing bacterial
182 (HUB) counts ranged from 4.2 x 10^4 to 6.4 x 10^5cfu/g and hydrocarbon utilizing fungal (HUF)
183 counts ranged from 1.5 x 10^3 to 4.0 x 10^3cfu/g. The results of soil samples taken 20m away from
184 the Pot location ranged from 7.0 x 10^5 to 8.2 x 10^6cfu/g for total heterotrophic bacterial counts,
185 fungal counts ranged from 2.3 x 10^3 to 1.5 x 10^4cfu/g, HUB ranged from 4.7 x 10^4 to 5.7 x
186 10^5cfu/g and HUF ranged from 2.0 x 10^3 to 3.5 x 10^3cfu/g. Also, the results of total heterotrophic
187 bacterial counts for Station 2 ranged from; 4.3 x 10^5 to 3.3 x 10^6cfu/g, fungi 2.0 x 10^3 to 3.3 x
188 10^4cfu/g, HUB ranged from 3.8 X 10^4 to 5.4 x 10^4cfu/g and HUF 1.6 x 10^3 to 3.5 x 10^3cfu/g,
189 while 20m away from the Pot total heterotrophic bacteria ranged from 1.3 x 10^7 to 6.5 x
190 10^7cfu/g, fungi 5.8 x 10^3 to 1.4 x 10^4cfu/g, HUB 5.4 x 10^4 to 1.1x 10^5cfu/g and HUF 3.1 x 10^3 to
191 4.7 x 10^4cfu/g. While the control samples taken from inside the community where no such

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activity is on, ranged from 2.6 x 10^{7} to 7.9 x 10^{7}cfu/g for total heterotrophic bacteria counts, total heterotrophic fungal counts ranged from 2.8 x 10^{4} to 5.3 x 10^{4}cfu/g, HUB 2.0 x 10^{2} to 3.1 x 10^{2}cfu/g and HUF 2.0 x 10^{1} to 2.3 x 10^{1}cfu/g. Mean values of counts are showed in Table 1. Figures 1 and 2 reveals the distribution of the various group of organisms identified during the period. In this study, twelve bacterial genera identified from the control site include: *Bacillus, Alcaligenes, Flavobacterium, Acinetobacter, Pseudomonas, Micrococcus, Proteus, Serratia, Enterobacter, Streptococcus, Escherichia*, and *Staphylococcus*. The following eight fungal genera were identified form the control: *Penicillum, Aspergillus, Fusarium, Mucor, Rhizopus, Geotrichum, Candida*, and *Cladosporium*. The most predominant bacterial species in the uncontaminated soil sample were *Bacillus, Acinetobacter,* and *Pseudomonas* species. The hydrocarbon utilizing bacteria from the control site include: *Bacillus, Pseudomonas,* and *Serratia* species. In this study, the microbial diversity between the uncontaminated soil and the contaminated soil samples were recorded (Table 2). In Station 1, the bacteria isolated around the Pot were; *Flavobacterium* sp, *Micrococcus* sp, *Bacillus* sp, *Pseudomonas* sp and *Acinetobacter* sp; and *Penicillum* sp, *Mucor* sp, *Rhizopus* sp and *Aspergillus* sp were the fungi isolated around Pot 1. *Bacillus, Pseudomonas and Acinetobacter sp, Mucor, Rhizopus, Penicillum, Fusarium* and *Aspergillus* sp were isolated 20 meters away from Pot 1. Station 2 Pot 2, had the following bacterial genera: *Serratia, Micrococcus, Bacillus, Pseudomonas* and *Acinetobacter* whereas *Penicillum, Aspergillus, Rhizopus,* were the fungal genera. Except *Acinetobacter sp and Micrococcus sp,* bacterial genera isolated from Station 2, Pot 2 were also isolated 20 meters away from the Pot. Also *Penicillium, Mucor and Aspergillus* were the fungal genera isolated from Station 2 Pot 2, which was slightly different from *Mucor, Rhizopus, Penicillium and Aspergillus* sp isolated 20 meters away from the Station 2, Pot 2. The results of total hydrocarbon contents range from 106 – 281mg/kg across the sampling locations. The highest value of 281mg/kg was observed in the month of May around Pot 1, the least value of 106mg/kg was observed for Pot 2 in the month of July. It was observed that the concentration decreased across the stations during the sampling period. This may be due to surface runoff as a result of the rains since that is the peak of the rainy season. (If the total hydrocarbon content decreased due to runoff, one would expect that the total heterotrophic bacterial count would increase; any explanations why it isn't so?).

Discussion

The impact of illegal crude oil refining activities on soil microbes were determined by the enumeration of total heterotrophic bacterial, total heterotrophic fungal, hydrocarbon utilizing bacterial and fungal counts presented in Table 1. When the microbial population and diversity of the impacted area was compared with that of the control it was observed that the control had higher population and diversity. This observation could be attributed to the presence of vegetation cover, high nutrient content (especially nitrogen and phosphorus) as a result of decomposition of organic materials to release nutrients and other environmental factors required for the survival of these microorganisms in the soil. Counts observed in this study is similar to

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that obtained by previous researchers in contaminated soil Obire and Anyawu 2009. The total heterotrophic fungi and hydrocarbon utilizing fungal counts reported in this study are higher than those reported by Douglas and Green (2015) who worked on the microbial communities found in a diesel contaminated soil. The continuous refining activities releases crude oil and petroleum products into the soil, resulting in pollution which could be inhibitory to certain group of organisms while it becomes an enriched microbial community for the other group capable of survival in such contaminations (Obire and Anyanwu, 2009; Douglas, 2018). From the results obtained, it was observed that the differentdifference between the THBC and HUB was not significant which means that most of the organisms found in the contaminated locations are hydrocarbon utilizing microorganisms which are capable of using these contaminants as a source of carbon.

Soil has been reported as a suitable medium that aids the growth and survival of microorganisms, but the introduction of these contaminants retard the activities of these organisms, thus giving room to organisms that have the ability of metabolizing such products and limiting the growth of non-metabolizers of the products (Chikere and Ekwuabu, 2014). Lower microbial counts were observed in the samples around the pots from both stations. This observation apart from the contamination from the crude oil and petroleum products may be attributed to the heat used for the distillation process. This is in conformity with previous studies who have reported that temperatures that exceed 70-80ºC are capable of killing many soil microbes and that non-spore forming fungi will be killed at 70ºC (Pattison *et al*., 2009).

Results of this study also show that microbial diversity was also affected by the oil refining activity. Douglas (2018) has also reported that higher concentrations of the illegal refined crude oil deposit lead to a uniform reduction in species diversity and population of soil fungi over time. Thus, continuous dumping of *kpo-fire* residue into the terrestrial environment would impact negatively on the crucial role played by these groups of organisms in decomposition and interfere with other metabolic activities of the organisms in the environment.

The fungifungal isolates in this study have been reported by previous scholars to be capable of metabolizing or utilizing crude oil pollutants (Obire *et al.,* 2008, Douglas, 2018). Also, Obire and Anyanwu (2009), in a previous study of soil samples contaminated with crude oil had isolated fourteen fungi genera belonging to *Alternaria* sp., *Aspergillus* sp., *Cephalosporium* sp.; *Cladosporium* sp.; *Fusarium* sp., *Geotrichum* sp., *Mucor* sp.; *Penicillium* sp.; *Rhizopus* sp. *Trichoderma* sp., *Candida* sp., *Rhodotolura* sp., *Saccharomyces* sp. And *Torulopsis* sp from the control soil, with five hydrocarbon utilizing fungi identified out of the fourteen. But in this study, *Alternaria* sp, *Cephalosporium* sp.; *Geotrichium* sp.; *Rhodotolura* sp.; *Trichoderma* sp, and *Fusarium* sp were not identified. The hydrocarbon utilizing bacteria identified by this study has been shown to have the ability to utilize crude oil as carbon source (Chikere and Ekwuabu, 2014: Douglas and Green, 2015).

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271 **Conclusion**

272 This research has shown that the illegal crude oil refining activities has increased the quantities
273 of crude oil, petroleum products and residue (waste) into the soil environment, with the
274 accompanying heat, used for the distillation process greatly affecting both microbial load and
275 diversity in the soil environment. The refining activities could exert a negative impact on the
276 population, diversity as well as the activities of soil microorganisms. Since, the microbial
277 diversity is important for soil health, community structure and functions. Thus, the continuous
278 exposure of the soil to the indiscriminate illegal refinery activities, will not only hamper the
279 texture or structure of the soil but, would also lead to a decline in microbial populations which
280 could pose a serious threat to the food chain, decomposition, nutrient recycling, bioremediation
281 and the ecological balance.
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283 **Conflict of Interests**
284 The author(s) have not declared any conflict of interests.

285 **References**

286 American Public Health Association (APHA) (2008) Standard Methods for the Examination of
287 Water and Waste Water. 21st ed. Washington DC.
288

289 Atlas, R.M.leave a space between R. M. and Philp, J 2005should be in bracket Bioremediation:
290 applied microbial solutions for real-world environmental cleanup. American Society for
291 Microbiology(ASM) press, Washington, DC, pp 78-105.
292

293 Aparna C, Saitha P, Himabindu V, Alok B, Anjaneyulu Y (2010) No article topic or journal
294 name?

295 Barnett, H. L. and Hunter, B. B. (1972). Illustrated Genera of Fungi Imperfecti. 3rd Ed., Burgess
296 Publication Co.

297 Cheesebrough, M. (2000) District Laboratory Practice in Tropical Countries. Part 2, Cambridge
298 University Press, London, UK. Pp 143 – 156.

299 Douglas, S. I. and Green, D. I. (2015) Microbial Communities Found in Diesel Contaminated
300 Soil. International Journal of Physical & Applied SciencesItalicise. 2(4): 38 – 48.

301 Chikere, C. B. and Ekwuabu, C. B. (2014). Culture-dependent characterization of hydrocarbon
302 utilizing bacteria in selected crude oil-impacted sites in Bodo, Ogoniland, Nigeria. African
303 Journal of Environmental Science & Biotechnology. All journal names should be in italics 8(6):
304 401-406.

UNDER PEER REVIEW

305 Chikere, C. B., Okpokwasili, G. C. and Ichiakor, O.(2009) Characterization of hydrocarbon
306 utilizing bacteria in Tropical marine Sediments. African Journal of Biotechnology. 8(11): 2541-
307 2544

308 Douglas, S. I. (2018). Effect of illegally Refined Crude Oil (Kpo-Fire) Residue on Soil Fungi.
309 International Journal of current microbiology and Applied Sciences. 7(12):3309-3316.

310 Holt JG, Krieg NR, Sneath PHA, Stanley JT, Williams ST (full stops and spaces) (1994).
311 Bergey's Manual of Determinative Bacteriology. 9th Ed. Williams and Wilkins Company,
312 Baltimore, USA. pp. 71-561.

313 Kalantary, R. R., Mohseni-Banpi, A., Esrafile, A., Nasseri, S., Ashmagh, F.F., Jorfi, S. and
314 Jafari, M.(2014) Effectiveness of biostimulation through nutrient content on the bioremediation
315 of phenanthrene contaminated soil. Journal of Environmental Health Science and Engineering.
316 12(143): 1-9.

317 Kumar, M. and Khanna, S. (2010) Diversity of 16S rRNA and dioxygenase genes detected in
318 coal tar-contaminated site undergoing active bioremediation. Journal of Applied Microbiology
319 108: 1252 – 1262.

320 Mills, A. L., Breuil, C. and Colwell, R. R. (1978) Enumeration of Petroleum Degrading marine
321 and estuarine Microorganisms by most probable number Method. Canadian Journal of
322 Microbiology.12:234-248.

323 Njoku, J. D., Ebe, T. E. and Akachukwu, O. E. (2016). Analysis of Heavy Metal Contamination
324 by Artisanal Refining Plants in The Niger Delta Region, Southern Nigeria. British Journal of
325 Environmental Sciences. 4(3): 39-48.

326 Citation: Renner R. Nrior, Salome Ibietela Douglas and Martha Tamunoemi Ojongo, 2017.
327 "Effect of wastewater on biodegradability of drilling fluid", International Journal of
328 Development Research, 7, (12), 17872-17876.

329 Obenade, M, and Amangabara, G. T. (2014) Perspective: The Environmental Implications of Oil
330 Theft and Artisanal Refining in the Niger Delta Region. Asian Review of Environmental and
331 Earth Sciences, 1(2): 25 - 29

332 Obire, O. and Anyanwu, E. C. (2009) Impact of various Concentrations of Crude Oil on Fungal
333 Populations of Soil. International Journal of Science & Technology. 6(2): 211-218.

334 Okpokwasili, G. C. and Okorie, B. B. (1988), Biodegradation Potentials of Microorganisms
335 Isolated From car engine lubricating oil. Tribol International 21(4): 215 -220.

336 Parmar, T. K., Rawtani, D., and Agrawal, Y. K. (2016) Bioindicators: the natural indicator of
337 environmental pollution. *Frontiers in Life Science*, 9(2): 110 -118.

UNDER PEER REVIEW

338 Pattinson, G.Sspace., K.A. initials should go after the last name Hammill, B.G. Sutton and P.A.
339 McGee (2009) Simulated fire reduces the density of arbuscular mycorrhizal fungi at the soil
340 surface. *Mycological Research journal*, 103: 491-496.

341 United Nations Environmental Programme (UNEP) 2011. www.unep.org. Accessed 12 June,
342 2012.

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347 Table 1: Mean Microbial Counts from the Sampling Site

Sample Location	THBC(cfu/g)	THFC(CFU/G)	HUB(CFU/G)	HUFC(CFU/G)
Station 1(pot)	2.3×10^5	2.6×10^3	2.2×10^4	2.3×10^3
20m away	7.1×10^6	1.9×10^4	7.2×10^4	3.0×10^3
Station 2(Pot)	8.8×10^5	5.0×10^4	5.9×10^4	1.0×10^4
20m away	3.6×10^6	3.8×10^5	3.3×10^4	3.3×10^3
Control	7.8×10^7	4.1×10^5	2.7×10^2	2.2 x 10

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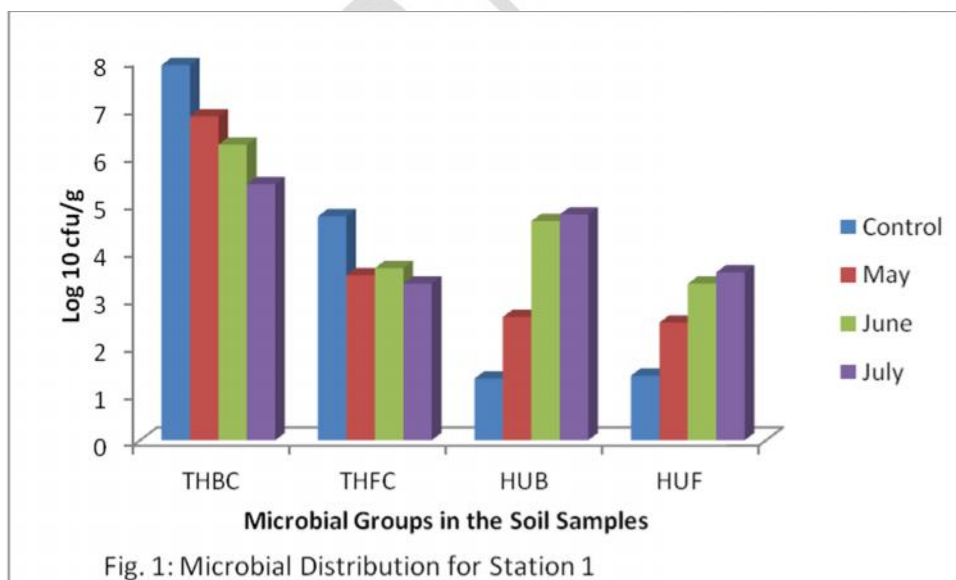

349 Fig. 1: Microbial Distribution for Station 1

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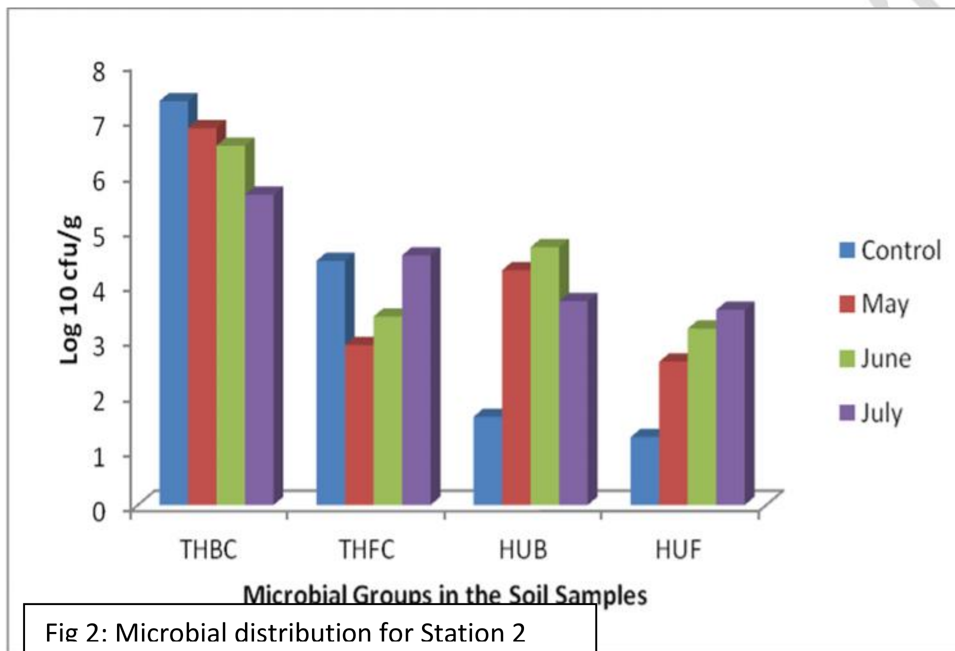

360 Fig 2: Microbial distribution for Station 2

361 Table 2: Microbial Diversity From the Sampling Locations

THB(control)	THB	HUB	THF	HUF
Bacillus sp	*Micrococcus* sp	*Pseudomonas* sp	*Aspergillus niger*	*Penicillium* sp
Klebsiella sp	*Bacillus* sp	*Micrococcus* sp	*Aspergillus flavus*	*Apergillus* sp
Pseudomonas sp	*Enterobacter* sp	*Acinetobacter* sp	*Cladosporium* sp	*Fusarium* sp
Serratia sp	*Micrococcus* sp	*Bacillus* sp	*Penicillium* sp	*Rhizopus* sp
Enterobacter sp	*Acinetobacter* sp	*Proteus* sp	*Fusarium* sp	*Mucor* sp
Micrococcus sp	*Flavobacterium* sp	*Serratia* sp	*Rhizopus* sp	*Cladosporium* sp

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Flavobacterium sp	*Serratia* sp	*Flavobacterium* sp	*Geotrichum* sp
Proteus sp	*Alcaligenes* sp		*Mucor* sp
Acinetobacter sp	*Proteus* sp		
Escherichia coli			
Alcaligene sp			
Streptococcus sp			

362 Key: THB total heterotrophic bacteria, HUB Hydrocarbon utilizing bacteria, hydrocarbon
363 utilizing fungi (HUF) should be here.

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