Impacts of Artisanal Crude oil Refining Activities on Soil Microorganisms

Abstract

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, HUB 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) and fungal (HUF) counts of the contaminated soil were enumerated. The mean total heterotrophic bacterial counts in Station 1 around the pot ranged from 2.5×10^5 to 1.8×10^6cfu/g, fungal counts ranged from 2.1×10^3 to 4.4×10^4cfu/g, HUB(HUB) counts ranged from 4.2×10^4 to 6.4×10^5cfu/g and hydrocarbon utilizing fungal (HUF) counts ranged from 1.5×10^3 to 4.0×10^3cfu/g. The results of soil samples taken 20m away from the Pot location ranged from 7.0×10^5 to 8.2×10^6cfu/g for total heterotrophic bacterial counts, fungal counts ranged from 2.3×10^3 to 1.5×10^4cfu/g, HUB ranged from 4.7×10^4 to 5.7×10^5cfu/g and HUF ranged from 2.0×10^3 to 3.5×10^3cfu/g. Also, the results of total heterotrophic bacterial counts for Station 2 ranged from; 4.3×10^5 to 3.3×10^6cfu/g, fungi 2.0×10^3 to 3.3×10^4cfu/g, HUB ranged from 3.8×10^4 to 5.4×10^4cfu/g and HUF 1.6×10^3 to 3.5×10^3cfu/g, while 20m away from the Pot total heterotrophic bacteria ranged from 1.3×10^7 to 6.5×10^7cfu/g, fungi 5.8×10^3 to 1.4×10^5cfu/g, HUB 5.4×10^4 to 1.1×10^5cfu/g and HUF 3.1×10^3 to 4.7×10^4cfu/g. While the control samples taken from inside the community where no such activity is on, ranged from 2.6×10^7 to 7.9×10^7cfu/g for total heterotrophic bacteria counts, total heterotrophic fungal counts ranged from 2.8×10^4 to 5.3×10^4cfu/g, HUB 2.0×10^2 to 3.1×10^2cfu/g and HUF 2.0×10^1 to 2.3×10^1cfu/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*. 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 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). 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 (Das and Chandran, 2011; Chikere et

al., 2011; Kostka et al., 2011). 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, Chikere *et al*., 2009). This research was carried out to evaluate the impact of the illegal crude oil refining activities on soil microorganisms. The Ke 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

The 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 as shown in Fig 1.

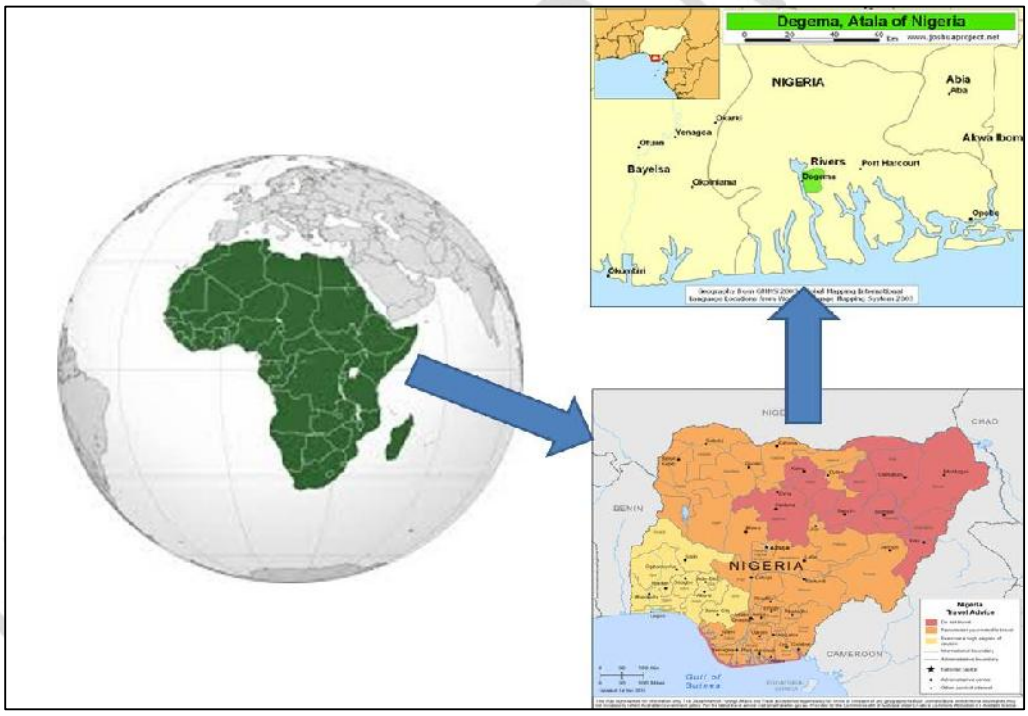

Fig 1 : location of study site in Ke, Degema Local Government Area, Rivers State, Nigeria.

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

To determine the total heterotrophic bacterial counts spread plate method was used 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 surface of the agar and evenly spread using a sterile hockey stick (spreader/inoculator). Plates were incubated at 30°C for 24 hours. The colonies that developed on the plates were counted and mean calculated for duplicate plates, results expressed in colony forming unit per gram (CFU/g) (Douglas and Green, 2015).

Enumeration of the Hydrocarbon Utilizing Bacterial Population

HUBpopulations in the soil samples were enumerated using mineral salt agar. The vapour phase transfer method using Mineral salt medium composition of Mills *et al*., 1978 was used as modified by Okpokwasili and Okorie (1988). Aliquot (0.1ml) of the 10^{-4} to 10^{-5} dilutions, previously obtained during the serial dilution of the soil samples, were inoculated in duplicates on appropriately labeled mineral salt agar plates which was freshly prepared and dried. The vapour phase transfer method in which a sterile Whatman No. 1 filter paper, placed on the lid of the Petri plate is saturated with 5ml Bonny light crude oil. Plates were inverted and incubated for 7days at 30°C. Colonies were counted after incubation, average counts calculated for duplicate plates and expressed as colony forming unit (CFU/g).

Enumeration of Total Heterotrophic Fungi

Spread plate method was used on Sabouraud Dextrose agar (SDA). An aliquot, 0.1ml of 10^{-3} and 10^{-4} dilutions were inoculated onto the freshly prepared SDA plates, in which 0.5% Ampicillin has been added. This was done to inhibit bacterial growth while allowing the growth of fungi (Cheesbrough, 2000). The inoculum was spread evenly using sterile hockey stick. Plates were inverted and incubated at 28^0C for 5days. Colonies that developed on the plates were counted, average counts on duplicate plates calculated and recorded as cfu/g.

Enumeration of the Hydrocarbon Utilizing Fungi

The MSA as composed by Mills *et al*., 1978 as modified by Okpokwasili and Okorie, (1988) to which 5% tetracycline was added to prevent bacterial growth was used. This medium was used for the isolation and enumeration of hydrocarbon utilizing fungi. From dilutions of 10^{-3} and 10^{-4}, 0.1ml aliquot was transferred on the freshly prepared plates; evenly spread using the hockey stick. The vapour phase transfer method in which a sterile Whatman No. 1 filter paper, placed on

the lid of the Petri plate is saturated with 5ml Bonny light crude oil. Plates were inverted and incubated for 7days at 30^{o}C. Colonies that developed on the plates after incubation were counted, average counts calculated for duplicate plates and expressed as colony forming unit (CFU/g) of soil.

Purification and characterization of Organisms

Discreet colonies that developed on the Nutrient and Mineral Salt agar plates were subcultured by streaking on Nutrient agar until pure cultures were obtained. Colonies on SDA and MSA for fungi were also subcultured by streaking on SDA until pure cultures were obtained. Pure cultures of bacterial and fungal isolates were preserved in bijou bottles containing nutrient and SDA slants respectively. The pure isolates were then refrigerated and were used for other analyses. The pure bacterial isolates were further investigated by carrying out routine microbiological analyses including; cultural and biochemical characteristics. The following test were done; Gram staining, cell motility, oxidase, indole and catalase production, citrate utilization, methyl Red-Voges Proskaeur test, acid/gas production from sugar fermentation, as described by Bergey's Manual for Determinative Bacteriology(Holt *et al*., 1994).

Identification of Fungal Isolates

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

Determination of Total Hydrocarbon Content (THC)

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

Results

The effect of illegal crude oil refining activities on soil microorganisms was investigated. 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, 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 10^4cfu/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 bacteria 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. Mean values of counts are showed in Table 1.

Figures 1 and 2 reveal 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 – 281 mg/kg across the sampling locations. The highest value of 281 mg/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.

Discussion

The impact of illegal crude oil refining activities on soil microbes were determined by the enumeration of total heterotrophic bacterial, total heterotrophic fungal, HUB and fungal counts

presented in Table 1. 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 (Gougoulias et al., 2014). Counts observed in this study is similar to that obtained by previous researchers in contaminated soil Obire and Anyawu 2009. 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 difference 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 fungal 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).

Conclusion

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

Disclaimer:

This paper is based on preliminary dataset. Readers are requested to consider this paper as preliminary research article, as authors wanted to publish the initial data as early as possible. Authors are aware that detailed statistical analysis is required to get a scientifically established conclusion. Readers are requested to use the conclusion of this paper judiciously as statistical analysis is absent. Authors also recommend detailed statistical analysis for similar future studies.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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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	2.2×10

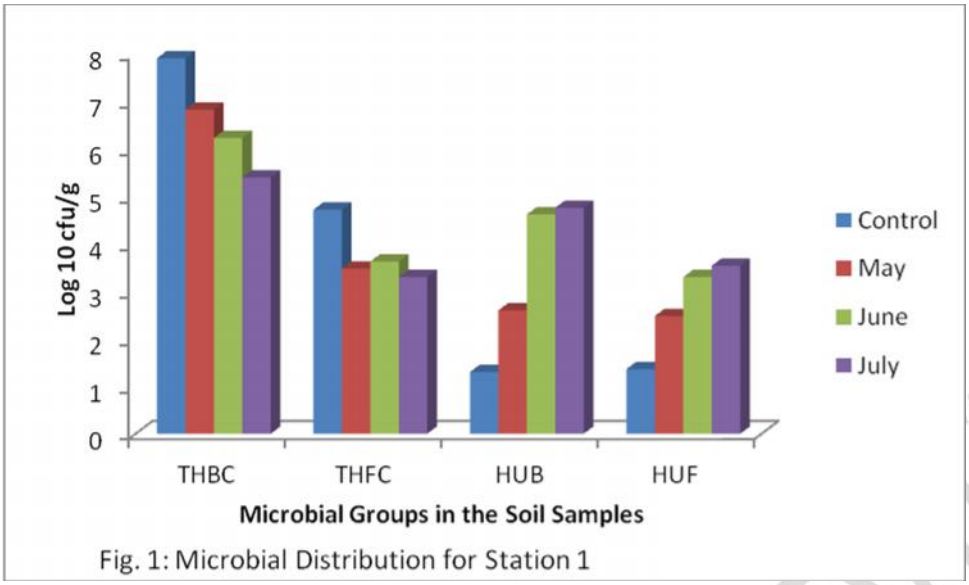

Fig. 1: Microbial Distribution for Station 1

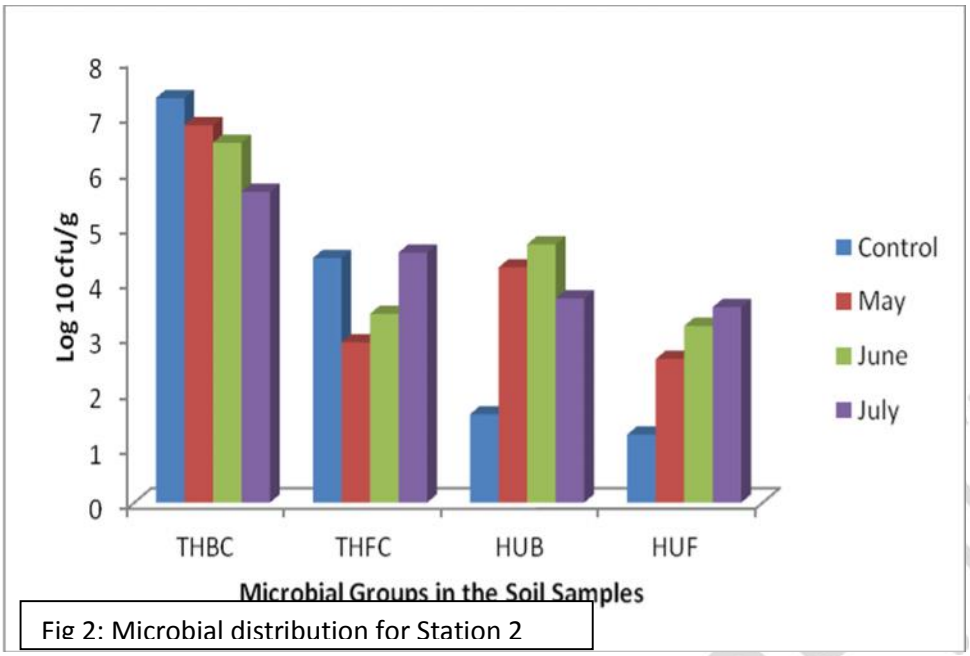

Fig 2: Microbial distribution for Station 2

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

Key: THB total heterotrophic bacteria, HUB Hydrocarbon utilizing bacteria, hydrocarbon utilizing fungi