European Journal of Applied Sciences – Vol. 13, No. 01
Publication Date: February 25, 2025
DOI:10.14738/aivp.1301.17980.
Iworiso, D. P., & Daokoru-Olukole, C. G. (2025). The Impact of Artisanal Refinery on Bacterial Community in the Soil from Selected Areas in Rivers and Bayelsa States, Nigeria. European Journal of Applied Sciences, Vol - 13(01). 352-371.
SERVICES FOR SCIENCE AND EDUCATION

The Impact of Artisanal Refinery on Bacterial Community in the Soil from Selected Areas in Rivers and Bayelsa States, Nigeria

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ABSTRACT

The soil, which is an intricate and dynamic biological system harboring a vast array of organisms, is directly affected by human activities as well as activities of illegal crude oil artisanal refineries. These activities have led to the introduction of hydrocarbons that range in high level toxicity and danger to human health. Hence creating problems for the ecological system. This study investigates the impact of artisanal crude oil refinery activities on soil bacterial communities in the Niger Delta regions, Okarki, Ododa (Rivers State), and Agba (Bayelsa State). Soil samples were collected and analysed following standard microbiological procedures, while the molecular method was used to characterize the bacterial isolates. Total petroleum hydrocarbon (TPH) concentration of soil samples was also analysed. The results of the bacteria count obtained from the soil samples for Total Heterotrophic Bacteria Count (THBC) ranged from 3.53 x 103 to 5.01 x 103 CFU/g, while Hydrocarbon Utilizing Bacteria Count (HUBC) ranged from 2.46 x 103 to 3.20 x 103 CFU/g. Total petroleum hydrocarbon (TPH) concentrations ranged from 107 to 7489 mg/kg around the refinery sites, which indicates soil contamination potential. The bacteria species identified were *Bacillus, Staphylococcus, Pseudomonans, Brucella*, *Acinetobacter, Enterobacter,* and *Enterococcus*. The molecular identification method reveals the following bacteria strains as Bacillus cereus, Brucella anthropi, and Stenotrophomonas maltophilia. The study emphasizes the intricate nature of soil microbial communities in artisanal refinery-affected regions and the need for long-term research to understand ecological impacts. It calls for effective mitigation strategies and stresses the importance of sustainable environmental management to address challenges posed by illegal artisanal crude oil refineries, advocating for a comprehensive approach.

Keywords: Artisanal crude oil refinery, Soil microbial communities, Niger Delta, Hydrocarbon contamination.

INTRODUCTION

In recent years, pollution from human activities has taken a toll on ecosystems, raising awareness about the need to protect and assess environmental damage (Obenade and Amengabara, 2014a; Naanen and Tolani, 2014). One significant concern is the pollution caused by oil refinery leaks, especially in Nigeria, a major oil producer in Africa (Asimiea and Omokhua, 2013; Nwankwoala *et al.,* 2017). Despite its vast oil reserves, Nigeria struggles to meet its

domestic demand for refined products and heavily relies on imports. This shortfall has led to the emergence of small-scale artisanal refineries, which use simple, cost-effective distillation processes to produce fuel and other products from crude oil (Akeredolu and Sonibare, 2015; Asuru and Amadi, 2016). However, these artisanal refineries, while economically attractive, have significant environmental impacts, including the release of crude oil and its derivatives into the soil ecosystem, affecting plants, animals, and microorganisms (Maclean and Steve, 2019).

The artisanal refinery operations, which often involve theft and illegal processing of stolen oil, have a direct impact on soil ecosystems, as many of these activities occur within this environment. This results in the release of crude oil and its derivatives, affecting the well-being of soil-dependent organisms (Douglas, 2018; Obenade and Amangabara, 2014a). While previous studies have discussed the refinery process's negative environmental and social outcomes, as well as its economic benefits (Obenade and Amengabara, 2014a; Naanen and Tolani, 2014), little attention has been given to how artisanal refineries influence soil microorganisms. This review aims to fill this knowledge gap by shedding light on the consequences of artisanal refinery operations on bacterial communities in the soil highlighting the need for a comprehensive understanding of these ecological impacts.

Statement of Research Problem

A threat to human health and agricultural practices, artisanal refineries (non-standardized refineries) have disturbed the activities of soil organisms (bacteria).

Aim and Objectives of the Study

Aim of the Study:

This study investigates the effects of artisanal refinery operations on the bacteria ecology in the soil.

Objectives of the Study:

The principal objectives of this study were to;

1. The main goal of this study was to determine the diversity and abundance of heterotrophic and hydrocarbon-using bacteria in soil contaminated by artisanal refineries.
2. Molecular analysis of isolates of bacteria from the research sites.
3. To ascertain the number of hydrocarbons from petroleum entering the soil that artisanal refineries have poisoned.

METHODOLOGY

Study Area

The study sites for the research project were situated in a region where illegal crude oil refining operations were actively occurring. These locations are in Ogbia Local Governemt Area (Agba) and River State in the Ahoada West local government area (Ododa and Okaki. They share a common area of vegetation. Ododa is situated next to a river in a forested area, rather far from the two other locations. Despite the fact that one flow pipe that crossed the vegetation supplied the crude to all three refinery locations.

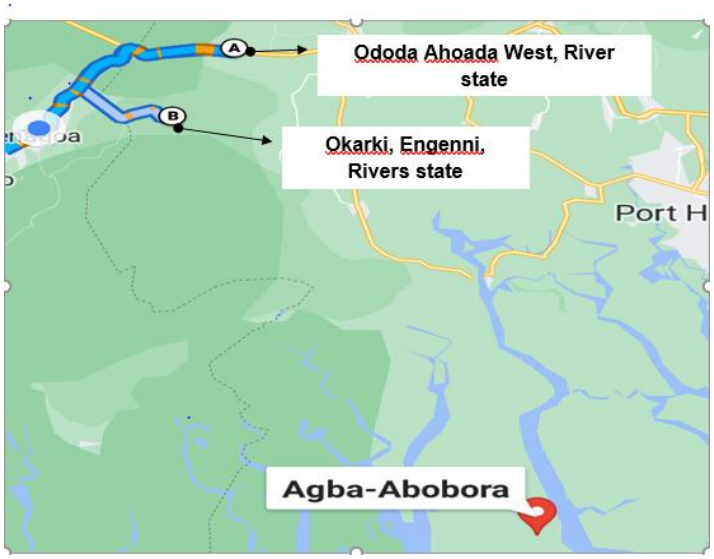

Figure 3.1 Map of study areas

Sample Collection

The central emphasis of this investigation revolved around soil samples. These soil samples were procured with a sterilized spatula from three distinct areas within the refinery site, and for each of these locations, five separate spots were sampled, employing sterile bags. At each site, soil samples were collected between 0-10 cm deep, with a 2-meter gap between each collection point. Additionally, a control soil sample was taken from locations 30 meters away from the refining site for comparison. Each soil sample was sent to the lab as soon as possible to enable analysis

Figure 3.2: Artisanal Refinery Site

Sterilization of Materials

The materials were rinsed with distilled water after being thoroughly cleaned with detergent and clean tap water, and then they were dried in an oven at 250^0 degrees Celsius for 45 minutes. After that, the materials were wrapped in aluminium foil and autoclaved to achieve sterility, which lasts for 15 minutes at 121^0 degrees Celsius.

Serial Dilution

Ten grams (10g) of soil were weighed out of the soil samples using a sterilized pipette, and they were then put into different conical flasks that had ninety milliliters of sterile normal saline. One milliliter was taken into a well labelled sterilized test tube containing 9mls of normal saline

making 10^{-1} dilution, 1ml from the 10^{-1} dilution was transferred into a second test tube making dilution 10^{-2} dilution. This procedure was repeated for test tube three through ten. After that, triplicate 0.1ml dilution of 10^{-3} 10^{-4} and 10^{-5} were plated to promote the growth of bacteria on appropriate media plate.

Analysis of Total Petroleum Hydrocarbon Content (TPH)

In accordance with the procedure outlined by Adesodun and Mbagwu (2008), a sample of the soil (5g) was combined with 25 ml of hexane and shaken with a mechanical shaker for 20 minutes to determine the total hydrocarbon content. After adding a Whatman (No. 1) filter paper to the final soilution, 1 millilitre of the extract was diluted with 50 milliliters of hexane to create the filtrate. Using n-hexane as the reference, the absorbance of this diluted soilution was measured at 460 nm using a HACH DR/2010 spectrometer.

Media Preparation

The preparation of the media used for the culture of organisms followed the manufacturer's instructions. Mineral Salt Agar and Nutrient Agar are the media that are utilized.

Isoilation Technique

Enumeration of Total Heterotrophic Bacteria:

The number of heterotrophic bacteria on nutritional agar was counted using the spread plate method. To make a homogeneous mixture, every single one-gram sample of soil was combined with nine milliliters of physiological saline. Next, 0.1 ml of each of the 10^{-3}, 10^{-4}, and 10^{-5} dilutions were applied equally to the agar surface utilising a glass spreader that has been sterilized. After that, those plates were allowed to incubate for 24 hrs at 35°C. After the incubation period, the number of bacterial colonies that had expanded on the plates was counted, and the average value across the three plates was computed. The method was supplied by Douglas and Green (2015), and as a result, the data were shown as colony-forming units per grams (CFU/g).

Enumeration of Hydrocarbon Utilizing Bacteria:

The number of hydrocarbon-using bacteria in the soil sample was counted using the vapor phase transference methodology on a mineral salt agar plate. The formulation of the medium was provided by Mills *et al*. (1978), and it was amended by Okpokwasili and Okorie (1988). One liter of pH 7.2 distilled water, 20 grams of agar, 0.8 grams of KH_2PO_4, 1.25 grams of Na_2HPO_4, 0.42 grams of $NaNO_3$, 0.29 grams of KCl, and 10.0 grams of NaCl were among the ingredients. Duplicate 0.1 ml samples were obtained from the 10^{-3} to 10^{-5} dilutions for each dilution by serially diluting the soil samples. These were inoculated to newly made, air-dried mineral salt agar plates after they had been appropriately labeled. The plates were incubated for one week at 30°C after being turned over. Following the incubation period, the colonies on the plates were counted, and the results, expressed as colony-forming units per grams (CFU/g), were obtained by averaging the counts from the three plates.

Characterisation of Microorganisms

By carefully streaking across nutrient agar repeatedly, bacterial colonies grown on mineral salt and nutrient agar plates were separated. This process was repeated until pure cultures were obtained. Bijou bottles fitted with nutrient agar and slants were used to preserve these clean

cultures. The uncontaminated isolates were kept for further investigation in a chilled setting using the methodology outlined by Cheesebrough (2000).

Identification of Bacteria

Following the guidelines of Bergey's Manual for Determinative Bacteriology Cheesebrough (2000), standard microbiological assays were used to examine the biochemical and cultural properties of the pure bacterial isolates.

Molecular Characterisation of Bacteria Isolate

Bacterial genomic DNA extraction:

The ZR bacterial DNA micro prep kit from Inqaba, South Africa, marked the beginning of DNA extraction. In a ZR Bashing Bead Lysis tube, 200 microliters of isotonic buffer were combined with dense bacteria isolates. Next, 750 microliters of lysis soilution were added, and the bead was beaten for five minutes at maximum speed. 400 microliters of supernatant were fed into a Zymo-Spin IV filter after centrifugation at 10,000 × g, and then the filter was spun again at 7000 × g. A 1600-microliter mixture was produced by adding 1200 microliters of DNA binding buffer. Of that, 800 microliters were put on a Zymo-Spin IIC column and spun at 10,000 × g, discarding the flow through. After washing and spinning, pre-wash and wash buffers were added, and 100 microliters of elution buffer were used to elude the DNA, which was then stored at -20 degrees Celsius.

DNA Quantification:

Nanodrop 1000 spectrophotometer was used to quantify DNA. Actions were made to initialise the sample with two microliters of sterile distilled water, blank it with normal saline, and place the sample (2 microliters) on the lower pedestal. To make contact, the taller pedestal was lowered, and the "measure" button was chosen to determine the concentration of DNA.

16S rRNA Amplification:

The isolates' 16s rRNA region was amplified for 35 cycles using primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and1492R (5'-CGGTTACCTTGTTACGACTT-3') in a 50-microliter reaction volume. The PCR mixture contained 0.4M primer concentration, MgCl, DNTPs, taq polymerase, and ×2 Dream Taq Master mix from Inqaba, South Africa. After five minutes at 95°C for denaturation, there were 35 cycles of cycling between 52°C and 72°C (30 seconds at each temperature), and then there was a five-minute extension at 72°C. Using a UV transilluminator, visualisation was done following separation on a 1% agarose gel at 120V for fifteen minutes.

Sequencing of 16S rRNA:

BigDye Terminator kit and 3510 ABI sequencers were used for sequencing at Inqaba Biotechnological in Pretoria, South Africa. 10 ul of BigDye sequencing buffer, 0.25 ul of BigDye terminator v1.1/v3.1, 10 uM PCR primer, and 2–10 ng of PCR template per 100 bp were used in the sequencing process. 32 cycles of 10 seconds of denaturation at 96°C, 5 seconds of annealing at 55°C, and 4 minutes of extension at 60°C comprised the sequencing conditions.

Phylogenetic Analysis:

In bioinformatics, data was modified using Trace Edit after sequence capture. Using BLASTN, more comparable sequences were obtained from NCBI. The resultant sequences were aligned using ClustalX. In MEGA 6.0, Saitou and Nei (1987) used the neighbor-joining approach to deduce the evolutionary lineage. A consensus tree with 500 replicates was used by Felsenstein (1985) to illustrate the evolutionary history of the taxa. Jukes and Cantor (1969) developed the Jukes-Cantor method to calculate evolutionary distances.

Statistical Analysis

Data analysis was carried out using SPSS 20.0. The significance of computed averages was assessed using the Duncan multiple test ($P = < 0.05$).

RESULTS AND DISCUSSION

Mean of Bacterial Count from Sampling Sites

The mean of bacterial count from three distinct sampling sites; Okarki, Ododa, and Agba shown in Table 4.1. The measurement unit for bacterial count is colony-forming units (CFU/g).

Table 4.1: Mean of Bacterial Count from Sampling Sites

Sample Location	THBC (CFU/g)	HUBC (CFU/g)
Ododa	5.01×10^{3a}	2.46×10^{3a}
Agba	3.53×10^{3b}	2.63×10^{3b}
Okarki	4.57×10^{3c}	3.20×10^{3c}

THBC – Total Heterotrophic Bacteria Count, HUBC – Hydrocarbon Utilizing Bacteria Count.

Investigating the Total Heterotrophic Bacteria (HUBC), Ododa exhibits the highest count with 5.01×10^3 CFU/g, I followed by Okarki with 4.57×10^3 CFU/g, and Agba with the lowest count of 3.53×10^3 CFU/g. This indicates that, in comparison to other places, Ododa exhibits a comparatively higher concentration of heterotrophic bacteria. About HUBC, or Hydrocarbon Utilizing Bacteria Count, Okarki exhibits the highest count of 3.20×10^3 CFU/g, followed by Agba with 2.63×10^3 CFU/g, and Ododa with the lowest count of 2.46×10^3 CFU/g. This implies that Okarki demonstrates a relatively greater concentration of hydrocarbon utilizing bacteria compared to the other locations. Conversely, Ododa displays the lowest count, suggesting a comparatively lower concentration of hydrocarbon utilizing bacteria.

Total Petroleum Hydrocarbon of Sampling Sites

Total petroleum hydrocarbons (TPH)I concentrations in milligrams per kilogram (mg/kg) at each of the five sampling places (places 1 through 5) and the control sample in Ododa, Agba, and Okarki are shown in Table 4.2. A total of eighteen (18) soil sample where collected from the three-refinery site and analysed. The findings show that there are distinct patterns and trends in TPH contamination at each of the locations. I

Table 4.2: Total Petroleum Hydrocarbon of Sampling Site

Sample Location	Ododa	Agba	Okarki
Spot 1	4317mg/kg	3051mg/kg	2314mg/kg
Spot 2	2306mg/kg	485.22mg/kg	600mg/kg
Spot 3	7489mg/kg	1346mg/kg	1180mg/kg
Spot 4	1087 mg/kg	5460 mg/kg	1421 mg/kg

Spot 5	3972 mg/kg	138.01 mg/kg	3527 mg/kg
Control	2035 mg/kg	1204 mg/kg	107 mg/kg

From the table shown above, Ododa TPH concentrations varied from 1087 mg/kg at Spot 4 to 7489 mg/kg at Spot 3. Spot 3 exhibited the highest concentration, suggesting a localized source of petroleum hydrocarbon contamination. The control sample in Ododa had a concentration of 2035 mg/kg, serving as a baseline for comparison against the contaminated spots.

Agba TPH concentrations ranged from 138.01 mg/kg at Spot 5 to 5460 mg/kg at Spot 4. Spot 4 displayed the highest concentration, indicating a notable presence of petroleum hydrocarbons in that particular area. The control sample in Agba had a concentration of 1204 mg/kg.

And in Okarki, TPH concentrations ranged from 600 mg/kg at Spot 2 to 3527 mg/kg at Spot 5. Spot 5 demonstrated the highest concentration, suggesting a relatively higher level of contamination compared to other spots within Okarki. The control sample in Okarki had a concentration of 107 mg/kg.

Ododaihad the greatest total contamination level when the TPH concentrations were compared across the locations; Spot 3 had the highest concentration at 7489 mg/kg. At Spot 4, Agba had the highest concentration at 5460 mg/kg, and at Spot 5, Okarki had the highest concentration at 3527 mg/kg. With respect to the lowest amounts, Agba (138.01 mg/kg at Spot 5) and Ododa (1087 mg/kg at Spot 4) showed the highest levels of contamination, with Okarki showing the least amount at 600 mg/kg at Spot 2.

Furthermore, within each location, there were notable variations in TPH concentrations across different sampling spots. In Ododa, concentrations ranged from 1087 mg/kg at Spot 4 to 7489 mg/kg at Spot 3. Agba exhibited a wider range, spanning from 138.01 mg/kg at Spot 5 to 5460 mg/kg at Spot 4. On the other hand, Okarkii demonstrated a narrower range, from 600 mg/kg at Spot 2 to 3527 mg/kg at Spot 5.

Bacterial Isolates from Sampling Sites

The results obtained from Table 4.3, 4.4, 4.5 and Fig 4.1 provides information on the bacterial isolates, respectively, obtained from the sampling sites. These isolates represent the microbial diversity present in the studied locations.

Table 4.3: Morphological and Biochemical Characteristics of Bacteria Isolate

S/No	Morphology on NA	Gram Stain	G^+/G^-	CAT	Oxi	CoA	Probable Organism
1	Smooth white to cream	Cocci	-v	+	-	-	*Acinetobacter sp*
2	White to cream and fairly circular in shape	Rod	+v	+	-	-	*Bacillus sp*
3	Opaque off white colour	Cocci	-v	+	+	-	*Brucella sp*
4	Greyish to white circular	Rod	-v	-	-	-	*Enterobacter sp*
5	White, moist and slimmy irregular shape	Cocci	-v	-	-	-	*Enterococci sp*
6	Fluorescent yellow to green	Rod	-v	+	-	-	*Pseudomonance*

7	Creamy white and appear in clusters	Cocci	+v	+	-	-	*Staphylococci*

SN = Serial Number, G^+/G^- = Gram Negative/ Gram Positive, CAT= Catalase Test, Oxi = Oxidase Test, CoA= Coagulase Test

Table 4.4: Bacterial Isolates from Sampling Sites

Sample Sites	Bacterial Isolates Identified
Ododa	*Brucella sp, Enterococcus sp, Pseudomononas sp., Bacillus sp., Staphylococcus sp.*
Agba	*Acinobacter sp., Pseudomonas sp., Bacillus sp., Staphylococcus sp.*
Okarki	*Enterobacter sp., Pseudomonas sp., Bacillus sp., Staphylococcus sp.*

Starting with the Ododa sample site, several bacterial isolates were identified (Tables 4.3, 4.4 and 4.5), including *Brucella sp., Enterococcus sp., Pseudomonas sp., Bacillus sp.,* and *Staphylococcus sp.* These bacterial isolates at the Ododa sample location point to a potentially varied microbial community that may be impacted by the surrounding environment and potential sources of contamination. In contrast, *Acinobacter* and *Enterobacter* had a negative occurrence, indicating that they were not found at the sampling site. Examining the percentage frequency of each bacterial isolate provides insights into the relative abundance of each organism compared to the total frequency. *Bacillus* had the highest percentage frequency with 35.7%, signifying its significant presence in the samples. *Staphylococcus* followed with a percentage frequency of 26.2%, indicating a relatively high abundance as well. *Pseudomonas*, *Brucella*, and *Enterococcus* had percentage frequencies of 16.7%, 11.90%, and 9.5%, respectively, suggesting their presence but at lower levels compared to *Bacillus* and *Staphylococcus*.

At the Agba sample site, the bacterial isolates identified in Tables 4.3, 4.4 and 4.5 include *Acinetobacter sp., Pseudomonas sp., Bacillus sp.*, and *Staphylococcus sp*. The shared presence of *Bacillus sp.* and *Staphylococcus sp.* across multiple sample sites may suggest their ubiquity in the sampled environments. The percentage frequency column provides a relative measure of each organism's abundance. *Bacillus* had the highest frequency at 32.1%, followed by *Pseudomonas* at 28.6% and *Staphylococcus* at 25%. *Acinobacter* contributed 14.3% to the total frequency.

Now, focusing on the Okarki sample site, the identified bacterial isolates are *Enterobacter sp., Pseudomonas sp., Bacillus sp.*, and *Staphylococcus sp*. The similarities and differences in bacterial isolates at the Okarki sample site suggest a unique microbial composition influenced by the specific environment, potential sources of contamination, and local factors. The percentage frequency column provides the relative abundance of each organism, as seen in Table 4.5. *Bacillus* had the highest frequency at 38.7%, followed by *Pseudomonas* at 32.3% and *Staphylococcus* at 19.4%. *Enterobacter* contributed 9.7% to the total frequency.

From the frequency distribution table showing the percentage frequencies of bacteria isolate of each sample sites, certain bacterial isolates are consistently found across different locations. *Bacillus sp., Pseudomonas sp.,* and *Staphylococcus sp.* are present in all three sample sites, indicating their potential role as part of the normal microbiota in these environments. However, each sample site also exhibits unique bacterial isolates, suggesting variations in microbial

composition based on the specific sampling location. The presence of specific bacteria, such as *Brucella sp.* in Ododa and *Acinetobacter sp.* in Agba, may be influenced by the specific environmental factors and sources of contamination in those particular locations.

Table 4.5: Percentage Frequency Distribution of Bacteria from Sampling Sites

	Ododa			**AGBA**			**Okarki**		
Organisms	**Occurrence**	**Frequency**	**(%) Frequency**	**Occurrence**	**Frequency**	**(%) Frequency**	**Occurrence**	**Frequency**	**(%) Frequency**
Bacillus	+	15	35.7	+	9	32.1	+	12	38.7
Enterococcus	+	4	9.5	-	0	0	-	0	0
Pseudomonas	+	7	16.7	+	8	28.6	+	10	32.3
Brucella	+	5	11.90	-	0	0	-	0	0
Staphylococcus	+	11	26.2	+	7	25	+	6	19.4
Acinobacter	-	0	0	+	4	14.3	-	0	0
Enterobacter	-	0	0	-	0	0	+	3	9.7
Total		42	100		28	100		31	100

Key: + = Positive/Present (growth), - = Negative/Absent (no growth), % = Percentage

The table shows the frequency of individual organisms (bacteria) randomly obtained from the growth on the cultured plates.

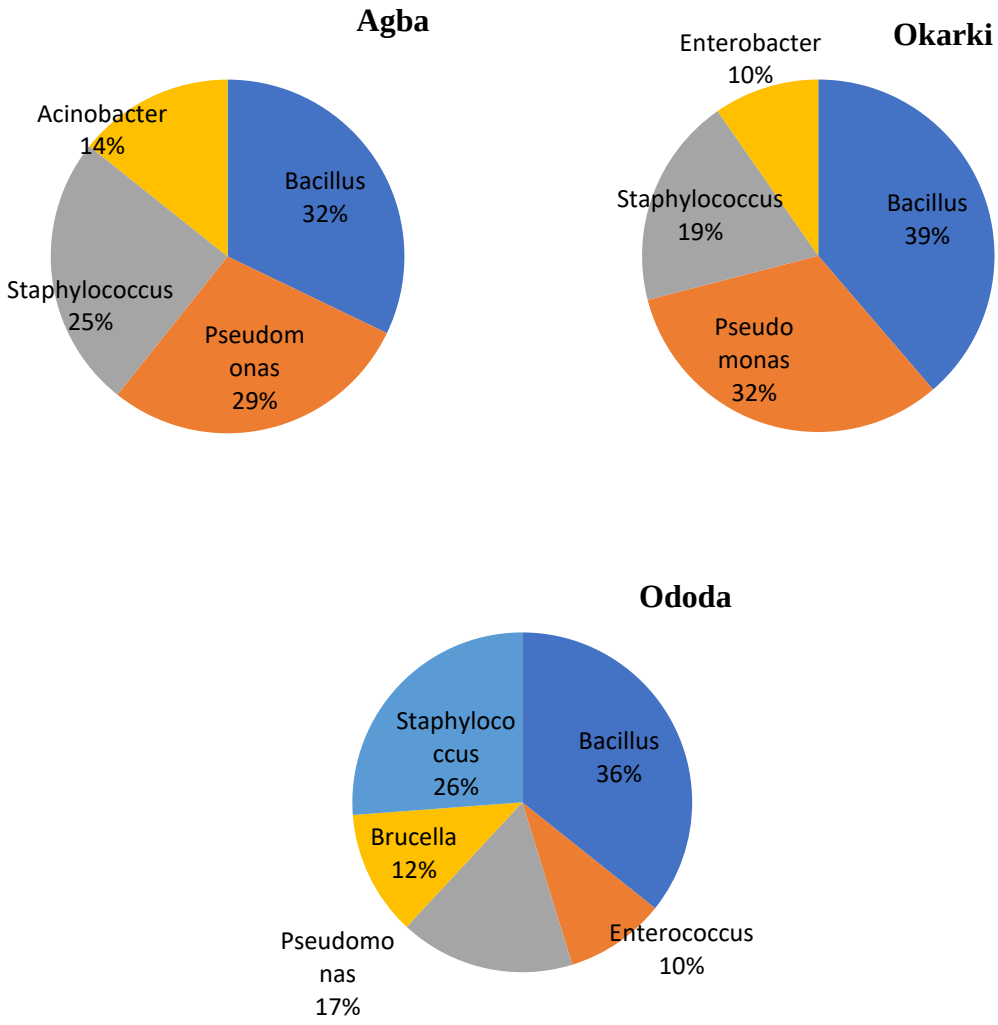

Fig 4.1. Percentage Frequency Distribution of Bacteria from Sampling Sites

Molecular Identification of Bacteria Isolates

Organisms are generally identified using conventional methods, such as morphological and biochemical methods. However, these methods lack credibility and have relatively few drawbacks, hence the need for conventional and molecular methods in this study. The molecular identification of the bacteria in this study is displayed in Table 4.6.

The results obtained by molecular characterization revealed bacteria such as *Brucella anthropi, Bacillus cereus,* and *Stenotrophononans maltophilia*.

Table 4.6: Phenotypic and Molecular Identification of Bacteria Isolates

Isolate Code	Phenotypic Identification		Molecular Identification
17	*Brucella sp*		*Brucella anthropi*
18	*Bacillus sp*		*Bacillius cereus*
111	*Pseudomonas sp*		*Stenotrophomonans Maltophilia*
112	*Enterobacter sp*		_____

113	*Acinotobacter sp*		_____
115	*Staphylococcus sp*		*Stenotrophomonans Maltophilia*
117	*Enterobacter sp*		*Brucella anthropi*

Bacterial Isolates:

For the isolate's 16s rRNA sequence, a mega blast search against the NCBI non-redundant nucleotide (nr/nt) database produced an exact match with very similar sequences. The isolates' 16S rRNA and that of other species showed 100% similarity. The isolates' phylogenetic placements within several bacterial species were consistent with the evolutionary distances determined by the application of the Jukes-Cantor technique, including *Brucella sp., Enterococcus sp., I Pseudomonas sp., Bacillus sp., Staphylococcus sp., Acinetobacter sp.*, and *Enterobacter sp.* Additionally, the analysis revealed close relatedness to *Bacillus cereus*, *Brucella anthorpi*, and *Stenotrophomonas maltophilia.* Figure 4.2

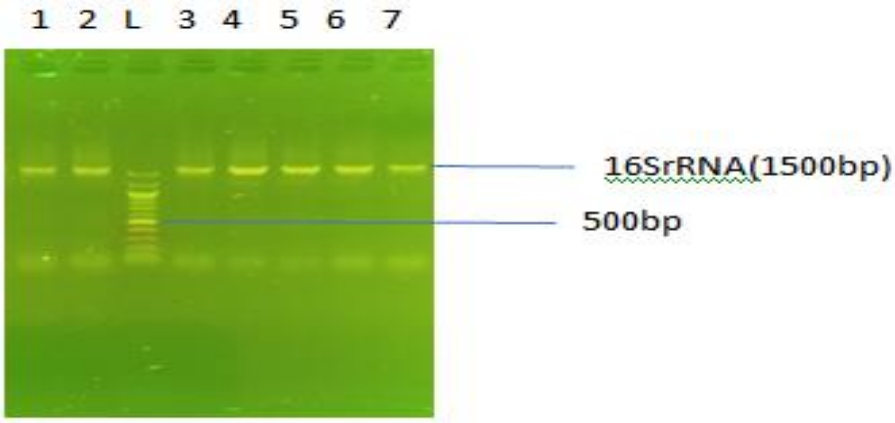

Plate 1: Visualization of Amplified 16S rRNA via Agarose Gel Electrophoresis: Lane Patterns and Molecular Ladder.

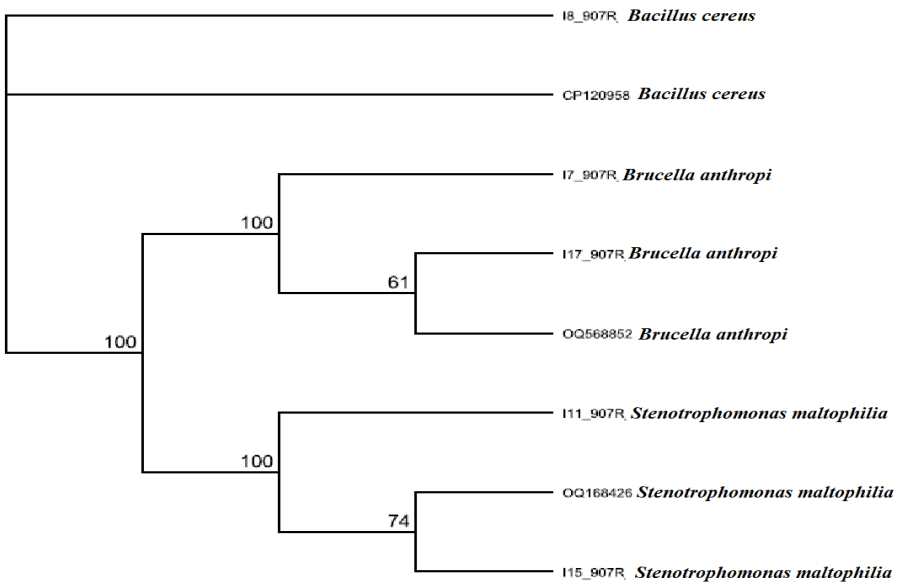

Fig 4.2: Phylogenetic Tree Revealing Evolutionary Distances Among Bacterial Isolates

DISCUSSION

Microorganisms are essential for determining the ecological health of both terrestrial and aquatic ecosystems due to their increased sensitivity to environmental changes and contamination, Parmar (2016). Since crude oil and its byproducts were released into the environment, robust microbial populations that could survive in contaminated environments have emerged. Chikere *et al*. (2009). Microbes exhibit remarkable adaptability, swiftly detecting and responding to even trace amounts of pollutants and ecological shifts. Parmar *et al*. (2016). In soil ecosystems, bacteria fulfill essential roles in the food chain, nutrient recycling, and biodegradation processes, aiding in the breakdown of organic matter and overall ecosystem functioning. Parmar *et al*. (2016). Monitoring and comprehending microbial communities provide valuable insights into pollution levels and ecosystem health, facilitating informed decision-making and effective environmental conservation measures.

This study aimed to determine the impact of artisanal refining processes on soil manly bacteria. The counts of all heterotrophic bacteria and all bacteria that use hydrocarbons, as the foundation for this investigation. The findings unveiled distinct variations in mean microbial counts among Okarki, Agba, and Ododa. Notably, Ododa exhibited higher bacteria counts, suggesting potentially greater microbial activity or biomass in comparison to Gaba and Okra. The observed differences in microbial counts across these locations could be attributed to diverse environmental factors, including soil composition, moisture levels, and organic material availably Obr and Aayanwu (2019) have smlary reports. The noteworthy average numbers of hydrocarbon-consuming bacteria (HUB) at every location should be emphasized, as this highlights the frequency and significance of bacterial contributions in the microbial ecosystems connected to artisanal crude oil refineres.

The findings highlight how artisanal crude oil refineries may affect soil microbial populations and cause differences in microbial counts in various settings. Ododa higher counts could be a sign of particular local circumstances, like heterorganic carbon content or exposure to crude oil leftovers, that support microbial growth. However, it is crucial to understand that this interpretation is based only on the average microbial counts that are presented on the table.

The microbial counts noted in this investigation are the culmination of diverse factors, encompassing the existence of vegetative cover, abundant nutrient content (notably nitrogen and phosphorus) stemming from organic matter decomposition, and a range of environmental circumstances pivotal for microorganism viably within the soil Gougoulas *et al.,* (2014). These outcomes harmonize with earlier studies centered on contaminated soil, showcasing resemblances in microbial counts Obrre and Anyanwu, (2009). Due to the persistent efforts of small-scale refineries, pollution is caused by the leakage of crude oil and petroleum products into the environment. This pollution produces an enhanced bacteria that can survive in such contaminated conditions, even though it may hinder some species of organisms (Obre and Anyanwu, 2009; Glas and betela, 2018).

Ogbona *et al.,* (2020) also reported similar microbial count that total heterotrophic bacterial count for uncontaminated soil was higher than that of the contaminated soil. Ogbona *et al.,* (2020) recorded (2.58 x 10^8cfu/g) for the uncontaminated soil and 2.1 x10^8 cfu/g for contaminated soil. n same van this research reveals higher heterotrophic bacterial count due to

activates of artisanal refining in soil environment thereby creating enrich or favorable conditions in the soil by the existing nutrients which serves as a platform for microorganisms to synthesize and utilize the nutrient as source of carbon and energy.

Although in this research three site where evaluated for mean microbial count and the overall, findings reveal variations n microbial counts among the sampled locations. Ododa consistently demonstrates higher bacteria counts across all categories, indicating a potentially elevated level of bacteria activity or a more favorable environment for microbial growth. Conversely, OOkark consistently exhibits the lowest microbial counts, while Agba falls in an intermediate position between Ododa and Okark. These variations may be influenced by environmental factors, nutrient availability, and specific human activates within each location. Gougoulas *et al.,* (2014).

The TPH values obtained from the sampling sites in this study demonstrate the presence of petroleum hydrocarbons in the soil. The results of this investigation, however, differ considerably from those of Douglas and Cornelus (2019). Lower TPH values, from 106 mg/kg to 281 mg/kg, were reported by Douglas and Cornelus (2019), with the greatest concentration recorded in May and the lowest in July. The surface runoff that happens during the wet season was blamed for the concentration drop. However, the TPH readings in this study are noticeably higher, suggesting a potential greater level of petroleum hydrocarbon pollution in the soil. n the same van the higher report in this research may be attributed to the sampling period as samples were obtained in the month of April were there was little or no rainfall as compared to the progressive increase in TPH values recorded in the month of July and May by Douglas and Cornelus (2019). Variations in sampling sites, ambient factors, and the particular sources of contamination could also be the cause of these discrepancies.

The overall, findings show that TPH pollution in Ododa, Agba, and Okark is spatially heterogeneous, with notable variations in contamination patterns and levels between the sites. The distribution and level of petroleum hydrocarbon contamination in the soil are shown by these data, which is helpful information for managing and accessing the environmental risks related to artisanal crude oil refining operations. Furthermore, the results suggest that the refinery operations at Ododa and Agba have led to higher levels of petroleum hydrocarbon pollution in the soil when compared to the control sites. On the other hand, compared to the control sample and the other sampling locations, Okark's petroleum hydrocarbon concentrations show that the impact of refining operations appears to be minor.

On the other hand, the dentification of different bacterial species provides insights into the microbial community composition in the soil samples. These findings indicate the presence of various bacteria with the potential to influence the ecological processes and dynamics of the studied environments. The diversity of bacteria soilates suggests a complex microbial ecosystem within the sampling sites. The findings in this research show smlartes and differences compared to the findings of previous studies. *Bacillus* and *Enterobacter* bacteria were also identified by Douglas and Cornelus, (2019) from artisanal crude oil-polluted soil, which is consistent with our study's findings. Similarly, Onwuna*et al.*, (2022) reported bacterial isolates in polluted soil samples, including *Enterobacter sp., Bacillus sp., Micrococcus sp., Staphylococcus sp.*, and *Pseudomonas sp.,* which partially correspond to some of the bacterial

isolates identified in our study. Species of *Bacillus*, *Staphylococcus,* and *Pseudomonans* have also been reported by Ogbonna *et al.* (2009) to demonstrate a significant numerical rise in hydrocarbon-polluted sites and as a primary degrader of petroleum hydrocarbons, which is supported by the findings of this current study. This is true even though the catalysts of intracellular enzymes produced by these organisms serves as the man mechanism by which microorganisms break down petroleum hydrocarbons. Xaokang *et al.,* (2009). t has been found that actinobacteria contain genes that both degrade and multiply aliphatic and aromatic hydrocarbon substrates with overlapping ranges. Funhoff and Van (2007).

Brucella anthropic was also isolated in this study as a hydrocarbon degrader. This also corresponds partially with the report by Muthukumar *et al.* (2003) that *Gallonella* degrades aliphatic protons, whereas *Brucella species* converts only aromatic rings to aliphatic protons, which are actually hydrocarbons. Juhasz *et al*. (2002) reported that *Pseudomonans maltophla*, the former name of *Stenotrophomonans maltophla*, was able to break down polycyclic aromatic hydrocarbons.

Furthermore, our investigation has previously demonstrated that the hydrocarbon-consuming bacteria may use crude oil as a carbon source. This ability is highlighted in the works of Douglas and Green (2015) as well as Chkere and Ekwuabu (2014).

Overall, the presence of certain bacterial isolates in our study aligns with previous findings, indicating the potential adaptability of these microorganisms to crude oil pollution. The differences observed could be attributed to various factors. Firstly, contamination sources associated with the refinery operations play a significant role. Artisanal refineries often release pollutants and waste materials into the environment, which can alter the soil conditions Douglas and Cornelus, (2019). These changes in soil pH, nutrient availability, and toxicity levels can selectively favor certain microbial species that are more adapted to these conditions Douglas and Cornelus, (2019). Because of the vast range of contaminants in terms of kind and concentration, the organization of the microbial community in the soil may vary amongst the sampling locations. Moreover, the characteristics of the soil have a major impact on the makeup of the soil microbial community Douglas and Cornelus, (2019). Variables such as soil type, texture, moisture content, and organic matter content might vary from one sampling site to another. Some bacteria species can tolerate certain soil properties to varying degrees. As a result, different microbial species might thrive in distinct biological niches created by the unique combinations of soil qualtes found at each site, leading to variations in the species composition Douglas and Cornelus, (2019)

Environmental factors also contribute to the differences observed in the microbial communities Gupta *et al.* (2017). Each sampling site may experience variations in climate, temperature, rainfall, and sunlight exposure. These environmental conditions can directly impact microbial growth and survival. Gupta *et al*. (2017). certain microbial species may be more adapted to specific environmental conditions, resulting in the presence of different species across the sites.

The microbial community fluctuations are also influenced by the proximity and interconnectivity of the sampling locations. Guo *et al* (2022). Microbes can spread through the movement of people and animals, as well as through the air and water. The exchange of

microbial populations may be impeded by obstacles or poor connectivity between the sites, resulting in different species assemblages at each site. Guo *et al* (2022). Moreover, the microbial communities may be shaped by past environmental influences and historical land use patterns (Jangd *et al.*, 2011; Osburn*et al*., 2021). One site may have a different microbial community than another, depending on past effects, history of exposure to contaminants, or particular land management techniques.

Lastly, time and the idea of ecological succession are important. According to Wrght *et al*., (2019), microbial communities are dynamic and change over time. The sampling locations might have been subjected to varying degrees of disturbance or established at distinct times following the artisanal refinery operations. certain temporal parameters can be used to explain variations in the species composition of the soil microbial communities. Wrght *et al.,* (2019).

Understanding the unique features of each site—such as the type of artisanal refinery operations, nearby land use practices, environmental factors, and the temporal dynamics of the microbial communities—is essential to comprehending the reasons behind the various species found in the soil microbial communities at the three sampling sites Idris *et al.,* (2018).

Considering the disparity in molecular and culture-dependent dentification

The isolates of bacteria that have been molecularly identified. For various reasons, distinct outcomes were found when the microbial community in the soil at sampling sites within the context of an artisanal refinery was identified using molecular and microbiological methods. Since Ogbonna *et al.* (2020) observed distinct bacterial species upon molecular analyses, the findings of this study on the molecular identification of the isolates are consistent with those findings. Ogbona *et al.,* (2020), reported *Nitrosomonas sp, Staphylococcus sp, Ctrobacter sp, Pseudomonas sp and Bacillus sp* as bacteria identified phenotypically. However, they recorded some differences when subjected to molecular analyses. From their report the individual organisms to their respective strains level by polymerase chain reaction (PCR), the bacteria isolates include *Comamonas testosterone, Staphylococcus saprophytcus, Chryseobarterum cucumers, Pseudomonas aurgnosa and Bacillus amylolquefacens* respectively*.*

In the same vain bacteria identified with traditional method in this research have distinct outcome in molecular identification. Seven bacteria generals were identified in this research which include *Brucella sp, Bacillus sp, Pseudomonas sp, Enterococcus sp, Acinotobacter sp, Staphylococcus sp and Enterobacter sp.* On molecualar characterization *Pseudomonas and Staphylococcus* were seen as *Stenotrophomonas maltophilia, Brucelar and Enterobacter sp* were reported as *Brucella anthropic, Bacillus* was recorded as *Bacillus cereus* although *Enterococcus spand Acinotobacter sp* had short sequence and were not sourcceful at the mega blast search.

One significant factor is the sensitivity and specificity of the identification methods employed. Literature has shown that molecular identification techniques, such as sequencing specific genetic markers like 16S rRNA, offer a higher level of accuracy and specificity compared to traditional culture-dependent identification methods.

By targeting specific genetic regions, molecular techniques can detect and identify a broader range of microorganisms, including those that are difficult to culture or have a typical growth conditions. This means that molecular identification may reveal microbial species that could have been missed by traditional methods, leading to differences in the identified microbial community.

The inability of culture-dependent identification to match molecular methods is partly due to the latter's capacity to identify non-viable or latent microorganisms Bornman and Triplett (1997). Even in latent or non-viable cells, microbial DNA or RNA can be found using molecular techniques. On the other hand, culturing methods, which could only be used to collect live, actively developing bacteria, are the basis of traditional microbiological identification. As a result, molecular identification can offer a more complete picture of the microbial community by incorporating inactive or non-viable species that might be important to the ecosystem but are missed by conventional techniques Torsvik and Ovreas (2002).

Furthermore, biases in sampling and culturing techniques can also contribute to differences in microbial identification. Traditional methods often involve culturing microorganisms in specific growth media, which can favor the growth of certain microbial species over others. Due to this bias in the culturing conditions, the microbial community included in the sample may not be fully represented. In contrast, molecular identification is not influenced by culturing biases and can provide a more unbiased assessment of the microbial community composition.

Moreover, molecular identification methods offer a higher resoilution and taxonomic depth compared to traditional methods Ovreas and Torsvik (1998). By sequencing specific genetic markers, molecular techniques can provide species-level identification and reveal subtle differences and variations within microbial communities. Traditional methods, on the other hand, often provide broader taxonomic classifications without reaching species-level resoilution. This difference in resoilution can lead to discrepancies in the identified microbial species between molecular and traditional methods.

Another reason could be the misidentification of microorganisms. In their study, Burdge *et al*. (1995) reported a case of misidentification, wherein *S. maltophilia* was erroneously dentified as *Pseudomonas cepacia*. About nine percent of the thirty-two clinical isolates were mislabeled as *P. cepacia* as a result of the oxidase test being read three minutes later than necessary and the DNase production tests not being watched for seventy-two hours before the results were verified. This misinterpretation holds clinical significance, particularly considering the importance of *P. cepacia*as a pathogen in patients with cystic fibrosis (CF). Analysing 16S rRNA gene sequences makes it easier to evaluate and determine the connections between different bacterial isolates. Species identification can be accomplished with partial 16S rRNA gene sequences, especially when compared to longer, closely related sequences. However, as Anoliefo *et al*. (2003) pointed out, whole 16S rRNA gene sequences are crucial for recognizing novel species. The 16S rRNA gene sequence, which is roughly 1550 base pairs long, provides a sufficiently unique measure. The results shown in agarose gel electrophoresis Plate 1 for the amplified 16S rRNA show distinct band pairings that relate to the specific bacteria that are being studied. Lanes 1–8 show the 16S rRNA bands at 1500 bp, while Lane L represents the 1000 bp molecular ladder.

The isolate's 16S rRNA sequence precisely matched extremely similar sequences in the NCBI non-redundant nucleotide (nr/nt) database, according to a megablast search. The 16S rRNA of the isolates showed complete species similarity. The Jukes-Cantor method's evolutionary distance calculations agreed with isolate 18 (907r)'s 16SirRNA's phylogenetic location within the *Bacillus species.* Notably, it demonstrated a closer affinity to *Bacillus cereus* (CP120958) than other *Bacillus species*. Isolate 17 (907r) displayed kinship with *Brucella anthropic* 117 (907r), and OQ568852 indicated proximity to *Stenotrophomonas maltophillia* 111 (907r), as ievidenced in Figure 4.2.

These results affirm the successful extraction of isolate DNA, submitted to GenBank (National Centre for Biotechnology Information, Maryland, USA), and subsequently assigned accession numbers.

SUMMARY, CONCLUSION AND RECOMMENDATION

Summary

The present study was undertaken to observe the impact of artisanal refining activities on soil bacterial community among three refinery locations. The impact was investigated and assessed by enumeration of hydrocarbon utilizing and heterotrophic bacteria. Total petroleum hydrocarbon concentration and microbial diversity was also determined. The investigation had it that the introduction of crude oil and petroleum product into the soil environment by the artisanal refining activities has resulted in the development of resilient microbial communities capable of surviving in contaminated conditions. Higher microbial activities or biomass in the soil where also recorded at the site where artisanal refining activities appear to be more. Monitoring and understanding the soil microbial communities provides valuable insights into the pollution levels and the well-being of the ecosystem enabling decision-making and appropriate environmental conservation measures.

Conclusion

The outcomes derived from this research underscore the definitive impact of ongoing illicit artisanal crude oil refining activities on the microbial community as well as plant and animal life.

The results of the investigation show that the presence of crude oil contamination in these areas has caused a significant increase in the total petroleum hydrocarbon (TPH) concentration in the soil. This increase is associated with increased toxicity, which interferes with the activities of organisms that are unable to metabolize hydrocarbons.

While the findings accentuate the adaptability and survival prowess of certain microorganisms in environments tainted by crude oil, they concurrently emphasize the multifaceted microbial assortment, showcasing the intricate resilience exhibited by the microbial community amidst the challenges posed by artisanal refinery undertakings.

Such findings propose plausible shifts in the microbial population and TPH contamination in close proximity to refinery sites. It is imperative to acknowledge the necessity for further research, aiming to holistically comprehend the enduringirepercussions of such activities on the soil ecosystem. This comprehensive understanding is vital to formulating robust and

sustainable environmental management and mitigation strategies, thereby effectively minimizing the ecological aftermaths associated with these activities.

Recommendation

After examining the impact of artisanal refineries on microbial community in the soil. the artisanal refinery have no concern with the chemical changes in the distillation processes there by leading to environmental waste in soilid, liquid and gaseous forms. Hence the suggestion that the artisanal refinery should clean up their operation exploring organisms that utilize hydrocarbon for sustainable bioremediation and mitigation processes. This will enhance the soil ability to carry out various functions as the soil relies on its physical, biological and chemical attributes to function.

Government policies should also be put in place to regulate artisanal refining activities.

Limitation of the Study

Obtaining the GPS location of the site was quite difficult as refinery operators could not allow the use of phones to assess the GPS location to avoid exposing their illegal refinery site to relevant environmental agencies and public.

Contribution to Knowledge

The understanding of the impact of artisanal refinery on soil bacterial community is significant most especially now that the nation Nigeria is facing environmental challenges as regard to environmental pollution. This research is significant as it has contributed to the knowledge of sustainable environmental and remediation effects, by comprehending the changes in microbial population and TPH contamination appropriate measures can be taken to minimize the ecological consequences and ensure the health and resilience of soil ecosystem to refinery affected areas.

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