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Assessing the Effects of Artisanal Refineries on Soil Fungal Communities in Niger Delta Region of Nigeria

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ABSTRACT

The alarming escalation of pollution across various ecosystems has underscored the critical importance of preserving environmental integrity and assessing the detrimental impact of contamination among the myriad forms of pollution. Oil pollution predominantly stemming from leaks and spills from oil refineries has garnered significant attention due to its widespread and deleterious consequence on soil ecosystem. This study was undertaken to observe the effects of artisanal crude oil refinery operations on fungal community in the soil from three locations namely Okarki, Ododa (Rivers State), and Agba (Bayelsa State) in the Niger Delta. About eighteen soil samples were collected and evaluated according to standard microbiological methods, and fungi isolates were characterized using the genomic approach. Additionally, the amount of total petroleum hydrocarbons (TPH) in soil sample concentration was evaluated. Total Heterotrophic Fungi Count (THFC) was 2.55 x 10^3 to 3.78 x 10^3 CFU/g, and Hydrocarbon Utilizing Fungi Count (HUFC) was 2.14 x 10^3 to 2.31 x 10^3. Within the refinery sites, total petroleum hydrocarbon (TPH) values varied from 107 mg/kg to 7489 mg/kg, suggesting a soil contamination from the artisanal refinery activities. About five fungi species were isolated on culture dependent methods and they include *Mucor, Penicillium, and Candida, Saccharomyces*, and *Aspergillu speices. Genomic* identification method revealed the following fungi strains: *Aspergillus sclerotorum, Aspergillus novoparasiticus, Aspergillus terreus, and Penicillium sp.* The study emphasizes the intricate nature of soil fungal communities in artisanal refinery-affected soil and the need to understand the ecological impacts on the soil after a long term artisanal activities. The complexity of fungi diversity observed in this study, revealed the resilience of the fungi community in the face of challenges posed by artisanal refineries.

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INTRODUCTION

The soil is an important habitat for both producers (green plants) and decomposers (bacteria and fungi) while air and water are both self- purifying systems with regard to most inorganic contaminants. soil is sink – receiving fall – out from the atmosphere which it absorbs or filters and could retain materials from infiltrating natural water.

Soil, which also serves as a natural recycling system, by providing habitat for a myriad of living organisms in the ecosystem. Soil microorganisms are involved in various biochemical processes and play a vital role in maintaining soil fertility and plant yield Nannipieri *et al.,* (2023). Changes in the activity and diversity of soil microbes may reflect changes in soil quality. It has been shown that different soil practices affected the structure and activities of soil microorganisms. Osburn *et al.,* (2019). Report by Iworiso and Daokoru-Olukole (2025) shows that artisanal crude oil refinery is well known for its significant source of soil contaminations due to lack of control. While contamination of soil has various origin whether anthropogenic or inherent to nature. Xiaokang and Chengtun*.,* (2019) report that contamination introduced by spillage resulting from pipeline ruptures, inadvertent leaks and inadequate handling at production sites affect nearby soil and water. The waste from these artisanal refinery sites is discharged directly into adjacent rivers and land without undergoing treatment causing significant harm to aquatic and terrestrial life (Iworiso & Daokoru-Olukole, 2025). Crude oil and petroleum product spills from artisanal refinery alter the microbial community organisational structure inside the soil environment reducing its diversity and increasing environmental stress (Maclean and Steve, 2019).

As a result of this crude oil contamination scientist have develop the interest to examining its detrimental effects on the environment. Chen *et al.,* (2005) looked at soil environmental quality and distribution of traces metal in surface soil., Nwankwoala *et al.,* (2017) focus on the effect of refinery activities on soil and water while (Obenade and Amengabara, 2014a; Naanen and Tolani, 2014) discussed the refinery process's negative environmental and social outcomes. little attention has been given to how artisanal refineries influence soil microorganisms. This research focus on the impact of artisanal refinery on fungal community in the soil aims to fill this knowledge gap by shedding light on the effect of artisanal refinery operations on soil fungal communities, revealing the need for a comprehensive understanding of these ecological effects. The activities of illegal crude oil refineries have negative effect on soil microorganisms.

The aim of this study is to investigate the impact of artisanal refinery activities on the fungal community in the soil, with the following objectives;

1. to determine the population and diversity of heterotrophic and hydrocarbon utilizing fungi in soil polluted by artisanal refinery.
2. genomic analysis of fungi isolates from the study sites.
3. to determine total petroleum hydrocarbon content of the soil polluted by artisanal refinery.

This study is critical to enable the understanding of fungi associated with crude oil contaminated soil and their diversity and to effectively stimulate reduction of the ecological consequences associated with artisanal crude oil refinery activities.

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 the Bayelsa State, Ogbia local government area (Agba) and River State, Ahoada West local government area (Okarki and Ododa). They are sited in a vegetation across a creek that is not far from the site where refinery runoff its discharged into the body of water. Agba and Okarki, share a common area of vegetation.

Sample Collection

Soil samples were the bases of this research work. These soil samples were procured from three distinct areas within the refinery site, and for each of these locations, five separate spots were sampled, employing sterile bags. At each individual spot, the soil samples were gathered from a depth of 0-10cm, maintaining a spacing of 2m between every collection point. Furthermore, a comparative control soil sample was gathered from positions located 30m apart from the refining site. Every soil sample was expeditiously conveyed to the laboratory to facilitate analysis.

Figure 3.1 Artisanal Refinery Site

Analysis of total Petroleum Hydrocarbon Content (TPH)

Soil sample (5g) was mixed with 25ml of hexane and shaken for 20 minutes using a mechanical shaker, following the method described by Adesodun and Mbagwu (2008). The resulting solution was then filtered through a Whatman (No.1) filter paper, and the filtrate was diluted by combining 1ml of the extract with 50ml of hexane. The absorbance of this diluted solution was measured at 460nm using an HACH DR/2010 spectrometer, with n-hexane used as the reference.

Enumeration of Total Heterotrophic Fungi

The Sabouraud Dextrose agar (SDA) was employed along with the spread plate methodology. A 0.1ml portion from the 10-3 to 10-5 dilutions was introduced onto freshly prepared SDA plates incorporating 0.5% Ampicillin, designed to hinder bacterial proliferation while facilitating fungal growth Holt *et al*. (1994). The inoculum was uniformly dispersed utilizing a sterile glass spreader. Subsequently, the plates were inverted and subjected to an incubation period of 5 days at 28°C. Upon completion of incubation, the colonies that emerged on the plates were tallied, and the average count for duplicate plates was computed and documented as cfu/g.

Enumeration of Hydrocarbon Utilizing Fungi

To isolate and enumerate hydrocarbon-utilizing fungi, a modified MSA medium was engaged, formulated based on Mills *et al*. (1978) with modifications according to Okpokwasili and Okorie (1988). To inhibit bacterial growth, 5% tetracycline was integrated into the medium. The constituents of the medium encompassed NaCl (10.0 g), $MgSO_4.7H_2O$ (0.42g), KCl (0.29g), KH_2PO_4 (0.83g), Na_2HPO_4 (1.25g), $NaNO_3$ (0.42g), agar (20g), and distilled water (1L), attaining a pH of 7.2. Portions of 0.1ml from dilutions spanning 10-3 to 10-5 were transposed onto recently prepared plates and uniformly disseminated through a glass spreader. The plates were subjected to the vapour phase transfer technique, thereafter inverted, and subjected to an incubation period of 7 days at 30°C. Subsequent to incubation, the colonies that flourished on the plates were counted, and the mean counts were computed for duplicate plates, expressed as colony-forming units (CFU/g) of soil.

Characterisation of Fungi

The fungal colonies observed on Sabourod Dextrose Agar (SDA) and Mineral Salt Agar (MSA) were sub-cultivated through streaking on SDA, repeating the procedure until pure cultures were attained. To conserve these uncontaminated cultures, bijou bottles equipped with SDA slants were employed. The uncontaminated isolates were stored in a refrigerated environment, preserved for subsequent analysis, following the approach described by Cheesebrough, (2000).

Identification of Fungi

To distinguish the fungal isolates, a comprehensive strategy encompassing both macroscopic and microscopic scrutiny techniques was employed. The macroscopic identification process entailed the examination of the distinct cultures' morphological features on the plates. Conversely, microscopy involved the extraction of a small sample from the culture, which was subsequently placed on a clean slide devoid of grease. Lactophenol blue was applied to the slide, creating a smear, and a cover slip was positioned over it. Subsequently, the slide was examined utilizing x10 and x40 objective lenses Holt *et al*. (1994). The observed attributes were meticulously documented and cross-referenced with the identification guide outlined in Barnett and Hunter, (1986).

Fungal Genomic DNA Extraction

DNA extraction was performed using the ZR fungal DNA mini prep extraction kit provided by Inqaba South Africa. A dense growth of pure fungal isolates was mixed with 200 microliters of isotonic buffer in a ZR Bashing Bead Lysis tube, followed by the addition of 750 microliters of

lysis solution. These tubes were securely placed in a bead beater equipped with a 2ml tube holder assembly and processed at maximum speed for 5 minutes. Subsequently, the ZR bashing bead lysis tubes were centrifuged at 10,000 × g for 1 minute.

The subsequent steps involved transferring 400 microliters of supernatant to a Zymo-Spin IV spin Filter (orange top) in a collection tube, which was then centrifuged at 7000 × g for 1 minute. Following this, 1200 microliters of fungal/bacterial DNA binding buffer were added to the filtrate, resulting in a final volume of 1600 microliters. Next, 800 microliters of this solution were transferred to a Zymo-Spin IIC column in a collection tube and centrifuged at 10,000 × g for 1 minute, discarding the flow through from the collection tube. The remaining volume was transferred to the same Zymo-spin and spun again.

In a new collection tube, 200 microliters of DNA Pre-Wash buffer were added to the Zymo-spin IIC, followed by centrifugation at 10,000 × g for 1 minute. Then, 500 microliters of fungal/bacterial DNA Wash Buffer were added, and the tube was centrifuged at 10,000 × g for 1 minute. The Zymo-spin IIC column was placed into a clean 1.5-microliter centrifuge tube. Next, 100 microliters of DNA elution buffer were added to the column matrix, and the tube was centrifuged at 10,000 × g for 30 seconds to elude the DNA. Finally, the ultra-pure DNA was stored at a temperature of -20 degrees Celsius for use in various downstream reactions.

Quantification of the extracted genomic DNA was carried out using the Nanodrop 1000 spectrophotometer. The initiation of the procedure involved a double-click on the Nanodrop icon, which led to the activation of the equipment's software. For blanking purposes, normal saline was employed, while 2 microliters of sterile distilled water were utilized for initialization. Subsequently, the extracted DNA (2 microliters) was positioned on the lower pedestal, and the upper pedestal was lowered to establish contact. The final step involved clicking the "measure" button, thereby ascertaining the DNA concentration.

Internal Transcribed Spacer (ITS) Amplification

For the amplification of the ITS region of the rRNA genes in the isolates, the ITS1F primer (5'-CTTGGTCATTTAGAGGAAGTAA-3') and the ITS4 primer (5'-TCCTCCGCTTATTGATATGC-3') were employed. Using the ABI 9700 Applied Biosystems thermal cycler, the PCR process was conducted with a final reaction volume of 50 microliters spanning 35 cycles. The PCR mixture consisted of the X2 Dream Taq Master mix supplied by Inqaba, South Africa, containing Taq polymerase, DNTPs, and MgCl, along with primers at a concentration of 0.4M, and the extracted DNA as the template. The PCR conditions included an initial denaturation step at 95ºC for 5 minutes, followed by denaturation at 95ºC for 30 seconds, annealing at 53ºC for 30 seconds, and extension at 72ºC for 30 seconds, repeated for 35 cycles. Subsequently, a final extension was performed at 72ºC for 5 minutes. The resulting PCR product was then separated on a 1% agarose gel at 120V for 15 minutes and visualized using a UV trans illuminator.

Sequencing of ITS

The sequencing procedure was conducted by Inqaba Biotechnological in Pretoria, South Africa, utilizing the BigDye Terminator kit on a 3510 ABI sequencer. Within a final volume of 10ul, the sequencing encompassed the following components: 0.25 ul of BigDye terminator v1.1/v3.1, 2.25ul of 5 × BigDye sequencing buffer, 10uM Primer PCR primer, and 2-10ng PCR template per 100bp. The sequencing conditions involved 32 cycles, with denaturation at 96°C for 10s, annealing at 55°C for 5s, and extension at 60°C for 4 minutes.

Phylogenetic Analysis

Following the sequence acquisition, the obtained data underwent editing through the bioinformatics algorithm Trace edit. Additionally, BLASTN was used to retrieve additional sequences sharing similarities from the National Center for Biotechnology Information (NCBI) database. These acquired sequences were then aligned using ClustalX. The evolutionary lineage was deduced using the Neighbor-Joining method in MEGA 6.0 Saitou and Nei (1987). To depict the evolutionary trajectory of the studied taxa, a consensus tree derived from 500 replicates was generated Felsenstein (1985). The Jukes-Cantor method Jukes and Cantor (1969) was applied for computing the evolutionary distances.

Statistical Analysis

Analysis of data was carried out using SPSS 20.0. Calculated means of data were tested for significant by the Duncan multiple test (P = ≤ 0.05).

RESULTS

The mean Fungi count from three distinct sampling sites; Ododa, Agba, and Okarki is shown in Table 4.1.

Table 4.1. Mean Fungi Count from Sampling Sites

Sample Location	THFC (CFU/g)	HUFC (CFU/g)
Ododa	$3.78\times10\text{^}3^{a}$	2.14×10^{3a}
Agba	$2.89\times10\text{^}3^{b}$	2.25×10^{3b}
Okarki	2.55×10^{3c}	2.31×10^{3c}

THFC - Total Heterotrophic Fungi Count, HUFC, HUFC - Hydrocarbon Utilizing Fungi Count.

When looking at the Total Heterotrophic Fungi Count (THFC), Okarki has the lowest count (2.55×10^3 CFU/g), Agba has the greatest count (2.89×10^3 CFU/g), and Ododa has the highest count (3.78×10^3 CFU/g). This suggests that OOdoda displays a relatively higher concentration of heterotrophicifungi compared to the other locations.

When examining the Hydrocarbon Utilizing Fungi Count (HUFC), the figures for Ododa, Agba, and Okarki are 2.14×10^3 CFU/g, 2.25×10^3 CFU/g, and 2.31×10^3 CFU/g, followed by, which are rather similar. Although minor differences exist, this indicates a similar concentration of heterotrophic fungi across the sampled locations.

Total Petroleum Hydrocarbon of Sampling Sites

Total petroleum hydrocarbons (TPH) concentrations n 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.

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.

Ododa had 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.iOn the other hand, Okarki demonstrated a narrower range, from 600 mg/kg at Spot 2 to 3527 mg/kg at Spot 5.

Fungal isolates

The results obtained from Table 4.3, 4.4, 45. And Fig 4.1 provides information on the fungi isolates, respectively, obtained from the sampling sites. These isolates represent the fungi diversity present in the studied locations.

Table 4.3. Macroscopic and Microscopic Characterization of Fung Isolate

S/No.	Colonial Macroscopic Characterization on SDA	Microscopic Characterization	Probable Organisms
1	Brownish black in colour, dark spores	Long smooth brownish condisphores attached to vesicle	*Aspergillus sp*
2	White background with black spores	Hyphae septate conidia globosed and attached to vesicle via sterigma	*Aspergilus sp*
3	Creamy colour, opaque, bacteria like growth is large	Positive to germ tube test	*Candida sp*
4	White mold	Hyphae non septate spirangiospores enclosed in a sporangium	*Mucor sp*
5	Light orange	Large gram positive oval spherical and elipsodal budding cell	*Saccharomyces sp*
6	Dark blue	Large gram positive oval spherical and elipsodal budding cell	*Saccharomyces sp*
7	Dark green in color, pale cream to yellow on revers blue grey at center	Septate hyaline hyphae simple conidiosphores, brush like conidia	*Penicillium sp*

Table 4.4 Fungal iIsolates from Sampling Sites

Sample Sites	Fungi Isolates Identified
Ododa	*Mucor Sp., Penicillium sp., Candida sp., Saccharomyces sp., Aspergillus sp.*
Agba	*Mucor Sp., Penicillium sp., Candida sp., Saccharomyces sp., Aspergillus sp.*
Okarki	*Mucor Sp., Penicillium sp., Candida sp., Saccharomyces sp., Aspergillus sp.*

Tables 4.3, 4.4, 4.5 and Fig. 4.2 display the fungal isolates found at the sampling locations. All three sample sites, Okarki, Agba, and Ododa, share the same fungal isolates: *Mucor sp., Penicillium sp., Candida sp., Saccharomyces sp.,* and *Aspergillus sp*. This implies that the soil at each of these locations contains a similar fungus community. For Okarki, *Mucor* had a positive occurrence with a frequency of 5 and a percentage frequency of 27.8%. *Penicillium* also had a positive occurrence with a frequency of 4 and a percentage frequency of 22.2%. *Candida*, *Saccharomyces*, and *Aspergillus* had lower frequencies but still showed positive occurrences.

In AGBA, Mucor had a positive occurrence with a frequency of 4 and a percentage frequency of 20%. *Penicillium* had a higher frequency of 6 and a percentage frequency of 30%. *Candida* and *Saccharomyces* had lower frequencies but still displayed positive occurrences. *Aspergillus* had a frequency of 6 and a percentage frequency of 30%. In Ododa, *Mucor* had a positive occurrence with frequency of 3 and a percentage frequency of 15%. *Penicillium* had a frequency of 5 and a percentage frequency of 25%. *Candida*, *Saccharomyces*, and *Aspergillus* had lower frequencies but still showed positive occurrences. *Aspergillus* had the highest frequency of 9 and the highest percentage frequency of 45%.

Table 4.5. Percentage Frequency Distribution of Fungi from Sampling Sites

	Ododa			AGBA			OKARKI		
Organisms	Occurrence	Frequency	% Frequency	Occurrence	Frequency	% Frequency	Occurrence	Frequency	% Frequency
Mucor	+	3	15	+	4	20	+	5	27.8
Penicillium	+	5	25	+	6	30	+	4	22.2
Candida	+	1	5	+	2	10	+	2	11.1
Saccharomyces	+	2	10	+	2	10	+	3	16.7
Aspergillus	+	9	45	+	6	30	+	4	22.2
Total			100		20	100		18	100

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

The table shows the frequency of individual fungi randomly obtained from the growth on the cultured plates.

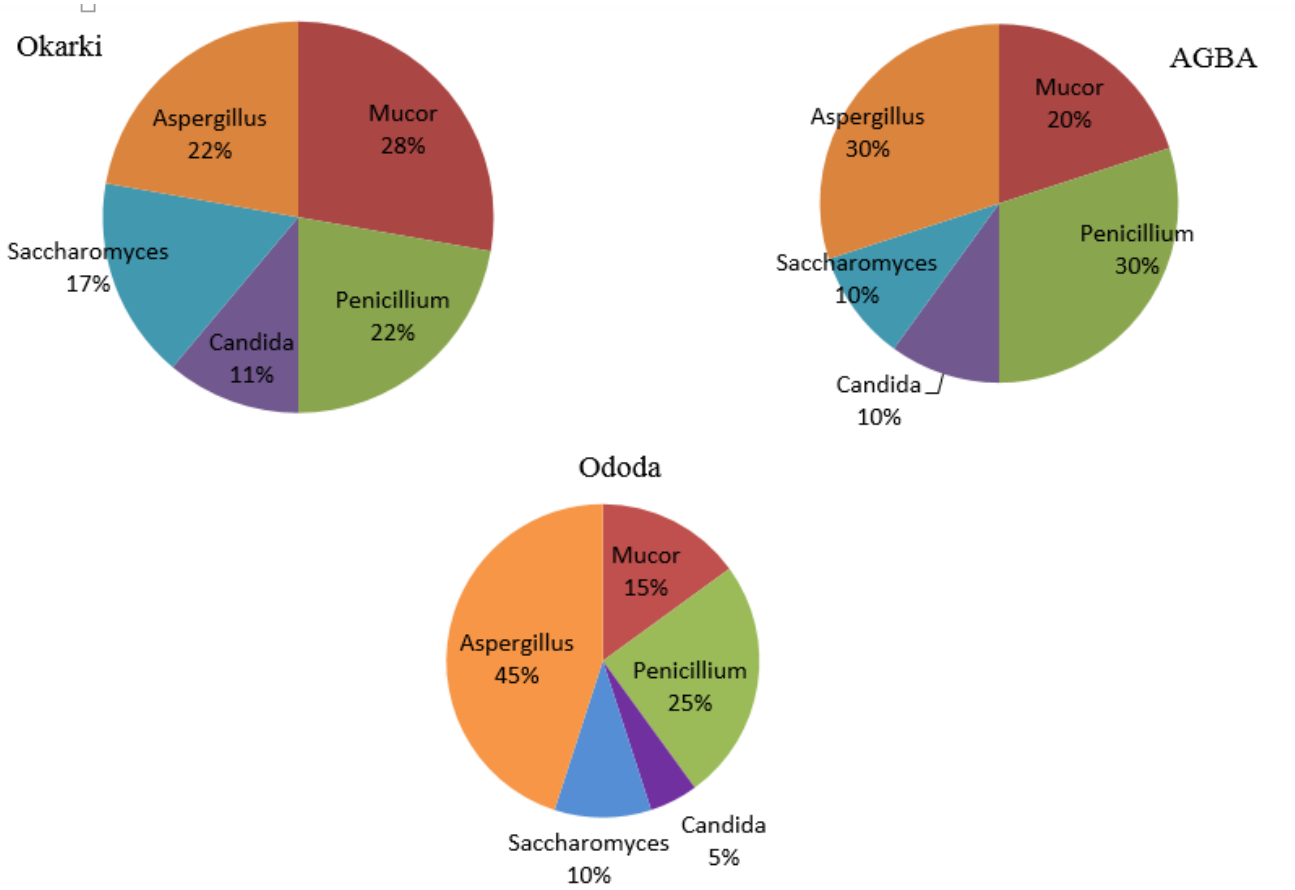

Fig 4.2. Percentage Distribution of Fungal isolates from Sampling Sites

Genomic Identification of Fungi 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 fungus in this study is displayed in Table 4.6. The results obtained by molecular characterization revealed three fungi belonging to *Aspergillus sp.,* such as *Novoparasiticus, Selerotorum,* and *Terreus,* and *Penicillium sp.,* were identified.

Table 4.6: Phenotypic and Molecular Identification of Fungi Isolates

Isolate Code	Phenotypic Identification	Molecular Identification
11	*Aspergillus sp*	*Aspergillus inovoparasiticus*
12	*Aspergillus sp*	*Aspergillus iselerotiorum*
14	*Saccharomyces sp*	*Aspergillus terreus*
15	*Peniicillium sp*	*Peniicillium sp*

Upon conducting a mega blast search in the NCBI non-redundant nucleotide (nr/nt) database, the ITS sequences derived from the isolates showed exact matches with sequences that were significantly similar. When compared to ITS sequences from other species, the isolates' sequences showed an absolute similarity rate of 100%. The isolates' ITS sequences revealed where they were phylogenetic among a variety of fungal species, and these matches with the evolutionary distances determined by the Jukes-Cantor method, encompassing *Mucor sp., Penicillium sp., Candida sp., Saccharomyces sp.*, and *Aspergillus sp.* Additionally, the analysis revealed close irelatedness ito *Aspergillus sclerotiorum, Aspergillus novoparasiticus, Aspergillus terreus, and Penicillium isp.* (Fig. 4.3).

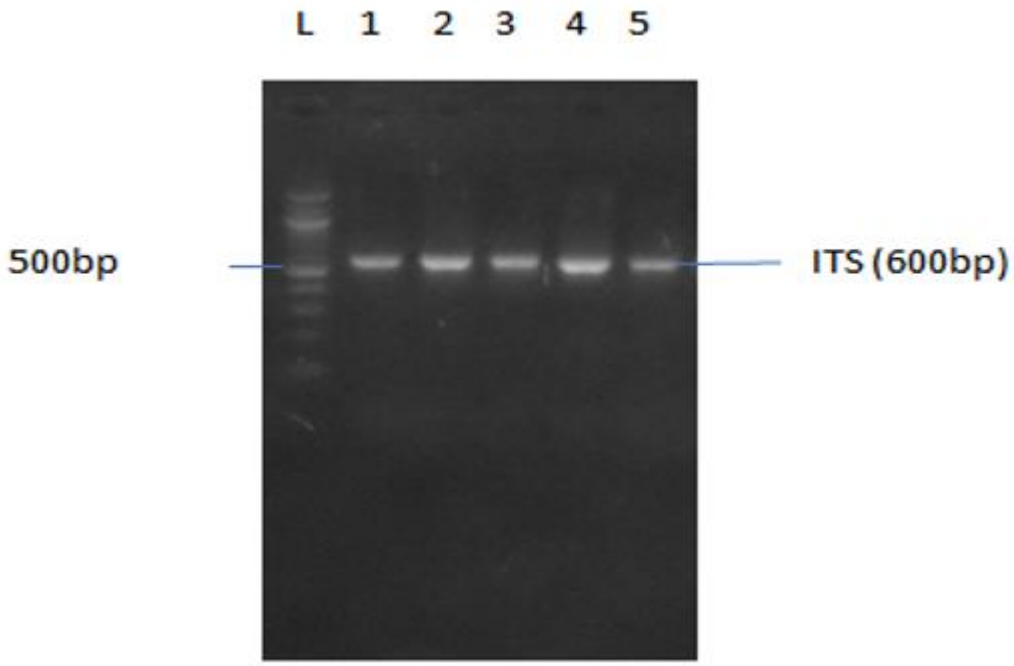

Plate 1: Visualization of Amplified ITS from Fungal Isolates via Agarose Gel Electrophoresis: Lane Patterns and Molecular Ladder

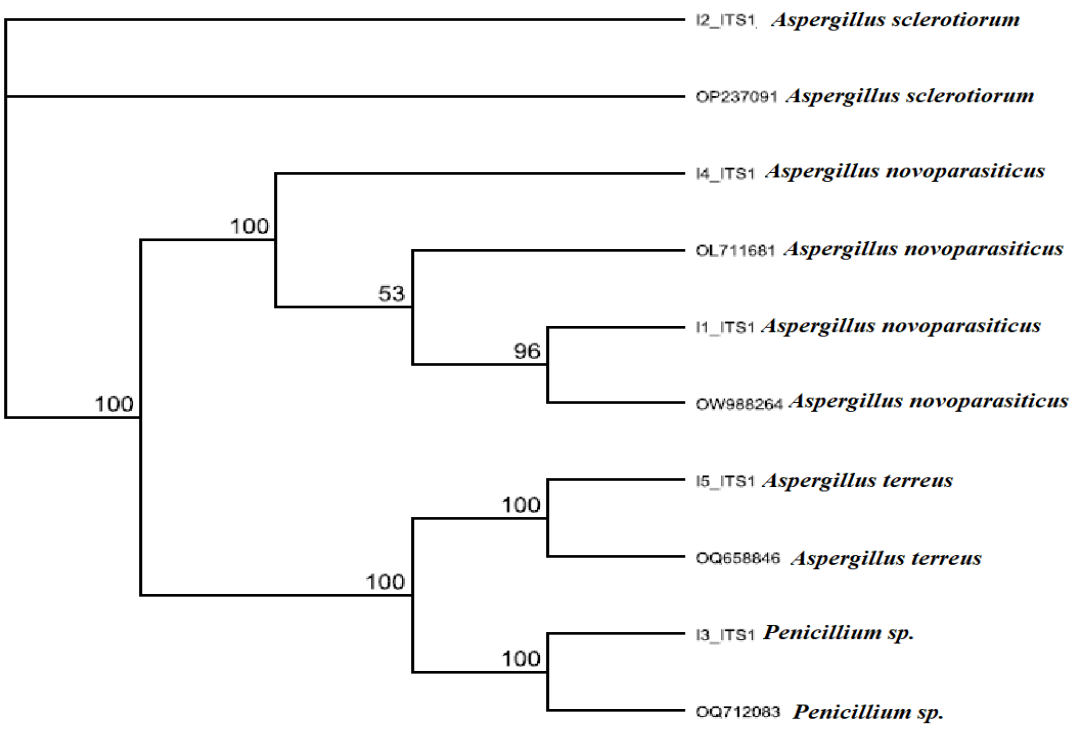

Fig 4.3: Phylogenetic Tree Illustrating Evolutionary Distances among Fungal Isolates

DISCUSSION

Microbes exhibit remarkable adaptability, swiftly detecting and responding to even trace amounts of

pollutants and ecological shifts. Parmar *et al*. (2016). In soil ecosystems, fungi communities 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). 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). Fungi 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).

Understanding the fungal communities will provide insights into pollution levels on the ecosystem, and this will enable the policy makers come up with an informed tdecision and consequently preserve the environments.

This research investigated the impact of artisanal refining processes on soil fungi. The findings unveiled variation in mean microbial counts among Okarki, Agba, and Ododa. Notably, Ododa exhibited higher microbial counts, suggesting potentially greater microbial activity or biomass in comparison to Agba and Okarki, Okarki consistently exhibits the lowest microbial counts, while Agba falls in an intermediate position between Ododa and Okarki. These variations may be influenced by environmental factors, nutrient availability, and specific human activities within each location. Gougoulias *et al.,*(2014).

The observed differences in microbial counts across these locations could be attributed to diverse environmental factors, including soil composition, moisture levels, and organic material availability. Obiri and Aayanwu (2019) have similary reports. The findings highlight how artisanal crude oil refineries may affect soil fungal populations and cause differences in microbial counts in various settings.

The fungal 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 viability within the soil (Gougoulias *et al.,*2014). These outcomes harmonize with earlier studies centered on contaminated soil, showcasing resemblances in fungal counts by Obrire and Anyanwu, (2009). Due to the persistent activities of these small-scale refineries, pollution is frequently caused through indiscriminate disposal of crude oil and petroleum products into the environment (Asimiea and Omokhua, 2013). This pollution produces an enhanced fungal community that can survive in such contaminated conditions, even though it may hinder some species of organisms (Obire and Anyanwu, 2009; Glas and Ibietela, 2018).

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 Cornelius (2019). Lower TPH values, from 106 mg/kg to 281 mg/kg, were reported by Douglas and Cornelius (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. in the same vain the higher report in this reseach may be attributed to the sampling period as samples were obtained in the month of April were there was little or no rainfall as comared to the progressive increase in TPH values recorded in the month of July and May by Douglas and Cornelius (2019). Variations in sampling sites, ambient factors, and the particular sources of contamination could also be the cause of these discrepancies.

The overall, ffindings show that TPH pollution in Ododa, Agba, and Okarki 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, theiresults 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, Okarki's petroleum hydrocarbon concentrations show that the impact of refining operations appears to be minor.

On the other hand, the identification of different ifungal species provides insights into the microbial community composition in the soil samples. These findings indicate the presence of various microorganisms with the potential to influence the ecological processes and dynamics of the studied environments. The diversity of isolates suggests a complex microbial ecosystem within the sampling sites. The findings n this research show similarities and differences compared to the findings of previous studies.

The presence of *Aspergillus sp., Saccharomyces sp., Candida sp., Mucor sp.,* and *Penicillium sp*. in our fungal isolates is consistent with the findings of (Glas and Ibietela 2018; Obire *et al.* 2008), who also discovered these fungi that can use or metabolize contaminants from crude oil in samples of contaminated soil. There is variance in both proportion and frequency even though the fungal isolates were identical throughout the sampling site.

However, there are differences between our study and previous findings. Obire and Anyanwu (2009) identified additional fungal genera in their study of crude oil-contaminated soil, including *Alternaria sp., Cephalosporium sp., Geotrichum sp., Rhodotolura sp., Trichoderma sp.,* and *Fusarium sp.,* which our investigation did not identify. This difference in fungal species composition could be attributed to variations in the specific locations, 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 Cornelius, 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 Cornelius, 2019) and environmental conditions between the studies.

Moreover, the characteristics of the soil have a major impact on the makeup of the soil microbial community (Douglas and Cornelius, 2019). Variables such as soil type, texture, moisture content, and organic matter content might vary from one sampling site to another. Some microbial 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 qualities found at each site, leading to variations in the species composition (Douglas and Cornelius, 2019)

Lastly, time and the idea of ecological succession are important. According to Wright *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. Wright *et al.,* (2019).

Conversely, the occurrence of these fungal isolates across all three sampling locations can be attributed to their ubiquitous nature within their surroundings Douglas (2018). These specific fungal species are recognized for their widespread distribution, being prevalent in various settings, encompassing soil, decaying organic materials, and indoor surroundings. They also display adaptability to diverse environmental circumstances, displaying endurance across a spectrum of pH levels and nutrient access. The resemblance between fungal isolates may also signify the existence of shared environmental elements and ecological niches. These fungal species exhibit the capability to harness a variety of organic substrates for growth and are acknowledged as opportunistic colonizers across heterogeneous environments. The availability of suitable organic substrates, moisture levels, and other environmental requisites conducive to their proliferation and persistence may be uniformly prevalent among the three sampling sites, Douglas (2018).

Considering the disparity in genomic and culture-dependent identification the isolates of fungus 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 fungal species upon genomic analysis. Finding from Ogbona *et al.,* (2020) also reported similarly for fungi species as *Rhizopus sp, Penicillium sp, Mucor sp* and *Fusarium sp* were dentified phenotypically but on molecular dentification *Rhizopus sp* was dentified as *Kodamaea ohmeri, Penicillium sp* as *Penicillium citrinum and brocae, Mucor sp as Lentinus Squarrosulus* and *Fusarium sp* as *Fusarium solani.*

The finding in this research also reported similar cases as *Apergillus sp, Saccharomyces sp, Penicillium sp* were recorded as *Aspergillus novoparasiticus,* and *Aspergillus sclerotiorium, Saccharomyces sp* as *Aspergillus terreus* and *Penicillium sp as Penicillium sp* on molecular characterization. Although *Mucor and* has short sequence and was also not successful on megablast search. One significant factor is the sensitivity and specificity of the dentification methods employed. Literature has shown that molecular identification techniques, such as sequencing specific genetic markers like ITS, 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 atypical growth conditions. This means that molecular identification may reveal microbial species that could have been missed by traditional methods, leading to differences in the dentified 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 (Gupta et al., 2017). 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 dentification 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 resolution 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 resolution. This difference in resolution can lead to discrepancies in the identified

microbial species between molecular and traditional methods.

Plate 1 shows the band pairings of every fungal isolate under examination in addition to the agarose gel electrophoresis data for the amplified ITS of the fungal isolates under inquiry. The 600 bps ITS bands are shown in lanes 1–5, while the 500 bps molecular ladder is shown in lane L.

The isolates' ITS and those of other species showed 100% percentage similarity. Jukes-Cantor evolutionary distance measurements concurred with the *Aspergillus genus's* ITS 12ITS1 phylogenetic order. Particularly, it demonstrated close affinity to *Aspergillus sclerotiorum* (OP237091) compared to other *Aspergillus species*. Isolate 14-ITS1 displayed close relation to *Aspergillus novoparasiticus* (OL711661), whereas *Aspergillus novoparasiticus* (OW988264) exhibited kinship with *Aspergillus terreus* (15ITS1). OQ658846 indicates proximity to *Penicillium species* (13-ITS1), as indicated in the phylogenetic tree. These results affirm the successful extraction of isolate DNA, submitted to GenBank (National Centre for Biotechnology Information, Maryland, USA), and subsequently assigned accession numbers.

The present study was undertaken to observe the impact of artisanal refining activities on soil microbial community among three refinery locations. The impact was investigated and assessed by enumeration of hydrocarbon utilizing and heterotrophic bacteria and fungi. 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 surving 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 results of this study showed the significant and damaging effects of illegal artisanal crude oil refining on the soil's fungal communities and, by extension, on plants and animals in the environment. The research reveals a substantial increase in Total Petroleum Hydrocarbon (TPH) levels in the soil, indicating higher toxicity levels due to crude oil contamination. While certain microorganisms (fungi) demonstrate their ability to thrive in such polluted environments, the study also underscores the complexity of fungi diversity, showcasing the resilience of the fungi community in the face of challenges posed by artisanal refineries.Therefore, based on the results of this study, we recommend that artisanal refineries should be discouraged in order to protect soil fungi communities.

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