CULTURE-DEPENDENT CHARACTERIZATION OF HYDROCARBON UTILIZING BACTERIA AND FUNGI FROM AN ARTISANAL CRUDE OIL REFINING SITE

Abstract

Crude oil refining is a global insert responsible for the production of a wide array of petroleum based products crucial for various sectors including energy, transportation, and manufacturing. However, the artisanal form of crude oil refining, often characterized by its small scale and limited technological infrastructure, has emerged as a major environmental concern in several regions around the world. Illegal refining of crude oil has led to oil spillages which in turn lead to a disruption of the microbial population of the soil. Hence, the aim of this study was to isolate and characterize the heterotrophic and hydrocarbon utilizing bacteria and fungi from an artisanal crude oil refining site. A total of thirty six (36) soil samples were collected from three (3) artisanal crude oil refining sites at iwofe, ogbogoro and ibaa community all in Rivers State, Nigeria. The samples were collected using soil auger and subjected to standard microbiological procedures such as culturing, isolation and identification. The results revealed significantly elevated levels of heterotrophic and hydrocarbon utilizing bacteria and fungi in the soil samples, with notable variations ($p \leq 0.05$) across the different artisanal crude oil refining sites. Total Heterotrophic Bacteria (THB) counts ranged from 3.75±0.07 in Iwofeto 2.70±0.14 in Ibaa (x10^5CFU/g), Total heterotrophic Fungal (THF) counts ranged from 2.80±0.14 recorded inOgbogoro to 1.45±0.07 recorded in Iwofe (x10^4CFU/g), Hydrocarbon Utilizing Bacteria (HUB) counts ranged from 2.25±0.21 in Ibaa to 1.30±0.14 in Iwofe (x10^3CFU/g) and Hydrocarbon Utilizing Fungi (HUF) counts ranged from 2.50±0.71 recorded in Ogbogoro to 1.40±0.14 recorded in Ibaa community (x10^2CFU/g) with significant differences ($p \leq 0.05$). Bacteria isolated and characterized from the soil include *Enterobacter*, sp, *Pseudomonasputida, Serratia*sp and *Bacillusamyloliquefaciens*while fungi include *Fusarium*sp, *Aspergillus*sp, *Penicillium*sp and *Mucor* sp. The microorganisms identified in this study have been reported to play key roles in the utilization of crude oil. *Pseudomonas*sp and *Bacillus*sp were the most dominant bacterial isolates and their dominance could imply that the soil could support the growth of crops. Thus, while they utilize the hydrocarbons, they also serve as plant-growth promoting microorganisms. Although some of the microorganisms in the present study have been reported to promote the growth of plant, the dumping of crude oil products legal or illegal should be minimized to avoid the extinction of plant-promoting microorganisms.

Key words: Crude Oil, Hydrocarbon, Artisanal, Refining, Dominance.

IntroductionhE

Crude oil refining is a globally significant industrial process responsible for producing a wide array of petroleum-based products crucial for various sectors, including energy, transportation, and manufacturing. However, the artisanal form of crude oil refining, often characterized by its small scale and limited technological infrastructure, has emerged as a major environmental concern in several regions around the world. Artisanal crude oil refining sites, commonly referred to as "illegal" or "bush" refineries, are prevalent in developing countries, especially in regions with abundant oil reserves but limited regulatory oversight and enforcement mechanisms (Akpan, 2017).These makeshift refineries, typically established in remote and economically disadvantaged areas, operate with rudimentary equipment and crude processing techniques, leading to a range of environmental challenges. One of the

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primary concerns associated with artisanal crude oil refining is the uncontrolled release of hydrocarbons and other toxic by-products into the surrounding soil, water bodies, and atmosphere. Such unregulated disposal practices pose severe risks to human health and local ecosystems, contributing to air and water pollution, soil degradation, and the disruption of natural habitats (Akpan, 2017; Nriagu, 2018).While the environmental impacts of artisanal crude oil refining have garnered significant attention from researchers and policymakers, the role of microbial communities, particularly hydrocarbon-utilizing bacteria and fungi, remains relatively understudied in these settings. Microorganisms are known to play pivotal roles in the natural attenuation and biodegradation of hydrocarbons in contaminated environments, exhibiting diverse metabolic capabilities that facilitate the breakdown of complex hydrocarbon compounds into less harmful substances (Atlas, 2010).

Material and Methods

Description of Study area

The study area was Rivers State, Nigeria. The study stations were artisanal crude oil refining sites in Iwofe and Ogbogoro in Obio Akpor Local Area as well as Ibaa in Emohua Local Government Area all in Rivers State, Nigeria.Table 1 shows the GPS Coordinates of the sample stations.

Table 1: Global Positioning System (GPS) Coordinates of the Sample Stations

Sample stations	GPS Coordinates
Iwofe	Latitude: 4° 48' 46.551
	Longitude: 6° 56 12.0906
Ogbogoro	Latitude: 4° 47 51.97956 N
	Longitude: 6° 58 49.7244" E
Ibaa	Latitude: 4° 4816.184" N
	Longitude: 6° 59" 1625

Sample Collection

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Soil samples were collected with the aidof a hand auger using the method by Pepper *et al.* (2015). A total of thirty-six (36) soil samples were collected once every month from the artisanal crude oil refining sites for a period of four (4) months. The samples were labeled and placed in sterile polythene bags and then transported to Microbiology Laboratory in Rivers State University, Port Harcourt for bacteriological analyses.

Inoculation and incubation of bacterialcultures

Inoculation of heterotrophic and hydrocarbonutilizing bacteria was done by asepticallytransferring aliquots (0.1 ml) of the dilution of $10^{-5}$and 10^{-3} onto properly driednutrients agar and mineral salt agar plates (containing fungosol 100 g (µ/ml) in duplicatesusing the spread plate method and they wereincubated at 35-37°C for 24 hours and 37°C for 3-5 days respectively (Chibuike *et al.*, 2023). Sub culturing of bacterialisolates was done to obtain pure cultures.Bacterial colonies were picked with sterileinoculating loop and streaked on freshlyprepared well dried nutrient agar (NA) plates (OkerentugbaandEzeronye, 2003).Colonial morphology such as shape, edge, color,elevation, surface, opacity and their consistencywere recorded. Biochemical assay was based onGram staining reaction, Motility, Catalase,Oxidase, Indole, Methyl red, Citrate, VogesProskauer, Starch hydrolysis and Sugarfermentation test.

Inoculation and incubation of fungalcultures

Inoculation of heterotrophic fungi andhydrocarbon utilizing fungi was also done byaseptically transferring aliquots (0.1 ml) of thedilution of 10^{-2} and 10^{-3} onto Sabourauddextrose agar (SDA) plates and Mineral Salt Agar (MSA)plates (containing chloramphenicol to suppressbacterial growth) in duplicates using spread platemethod. The inoculated plates were then incubated at 28±2°C for 5-7 days (Chikere and Ekwuabu, 2014). Fungalisolates from the heterotrophic and hydrocarbonutilizing bacteria populations were sub

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sub-cultured onto freshly prepared SDA plates.Fungal identification was based on macroscopicand microscopic characteristics.

Data Analyses

The data obtained was analyzed using analysis of variance (ANOVA) to test for significance and were differences occur, Duncan multiple range test was used to separate the means using the Statistical Package for Social Science (SPSS) version 25 (Bewick *et al*., 2004).

Results

Microbial Population of Crude Oil Polluted Soil

Results of the mean total heterotrophic bacteria (THB) and total heterotrophic fungi (THF) count across the artisanal crude oil refining sites are presented in Table 1.The THB count ranged from $2.70 \pm 0.14 \times 10^5$CFU/g (Ibaa) to $3.75 \pm 0.07 \times 10^5$CFU/g (Ogbogoro). Heterotrophic fungi count (THF) ranged from $1.45 \pm 0.07 \times 10^4$CFU/g (Ibaa) to $2.70 \pm 0.14 \times 10^4$CFU/g (Iwofe).There was a significant difference ($p \leq 0.005$) in the total heterotrophic bacteria and fungal counts in the soil across the artisanal crude oil refining sites. Table 2 presents the mean hydrocarbon utilizing bacterial and fungal(HUB and HUF) counts from the artisanal crude oil refining sites. Iwofe had the least HUB population ($1.30 \pm 0.14 \times 10^3$CFU/g) while Ibaa had the highest HUB population ($2.25 \pm 0.21 \times 10^3$CFU/g) across the artisanal crude oil refining sites. Hydrocarbon utilizing fungal (HUF) count ranged from $1.40 \pm 0.14 \times 10^2$CFU/g (Iwofe) to $2.50 \pm 0.71 \times 10^2$CFU/g (Ogbogoro).

Table 2:Mean Heterotrophic Bacterial and Fungal count from the Artisanal Crude Oil Refining Sites

SAMPLING SITE	THB (10^5)	THF (10^4)
Ibaa	2.70 ± 0.14^a	1.45 ± 0.07^a

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Iwofe	3.75±0.07^{b}	2.70±0.14^{b}
Ogbogoro	3.15±0.07^{c}	2.80±0.14^{b}

Key: THB: Total heterotrophic bacteria, THF: Total heterotrophic fungi

Table 3: Mean Hydrocarbon Utilizing Bacterial and Fungal count from the Artisanal Crude Oil Refining Sites

SAMPLING SITE	HUB (10^{3})	HUF (10^{2})
Ibaa	2.25±0.21^{a}	1.85±0.07^{a}
Iwofe	1.30±0.14^{b}	1.40±0.14^{a}
Ogbogoro	2.20±0.14^{b}	2.50±0.71^{a}

Key: HUB: Hydrocarbon utilizing bacteria, HUF: Hydrocarbon utilizing fungi.

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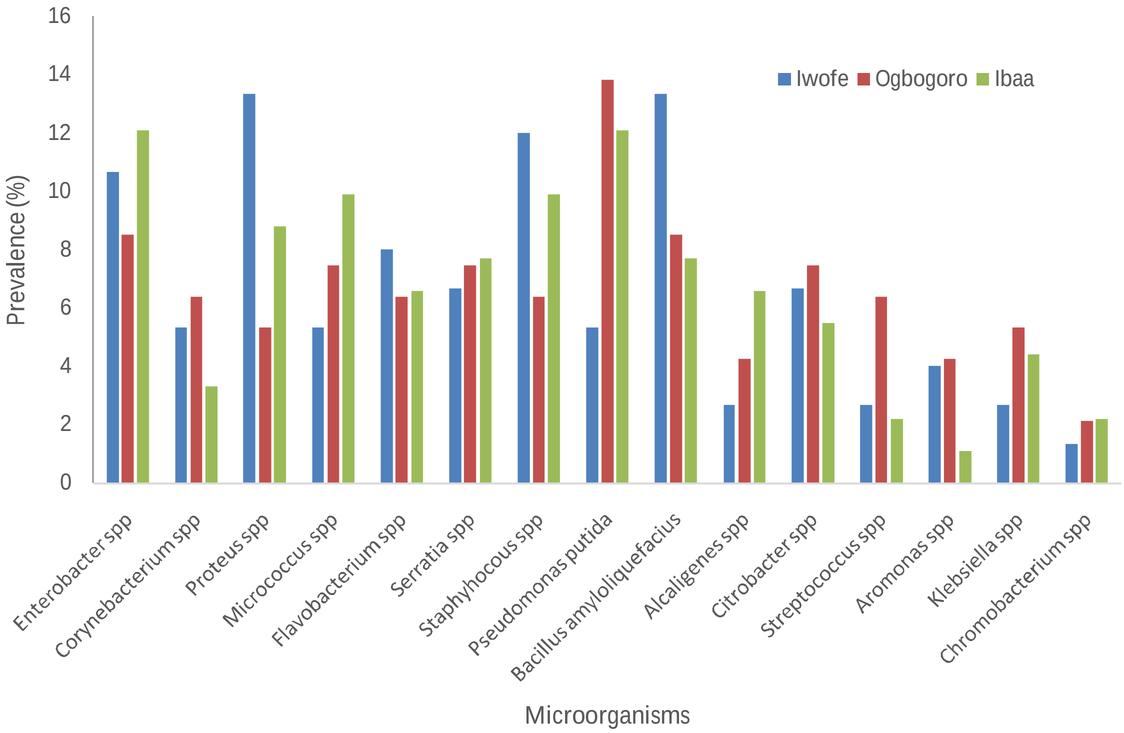

Fig. 1: Prevalence of the Bacteria Isolated from the Artisanal Crude Oil Refining Sites

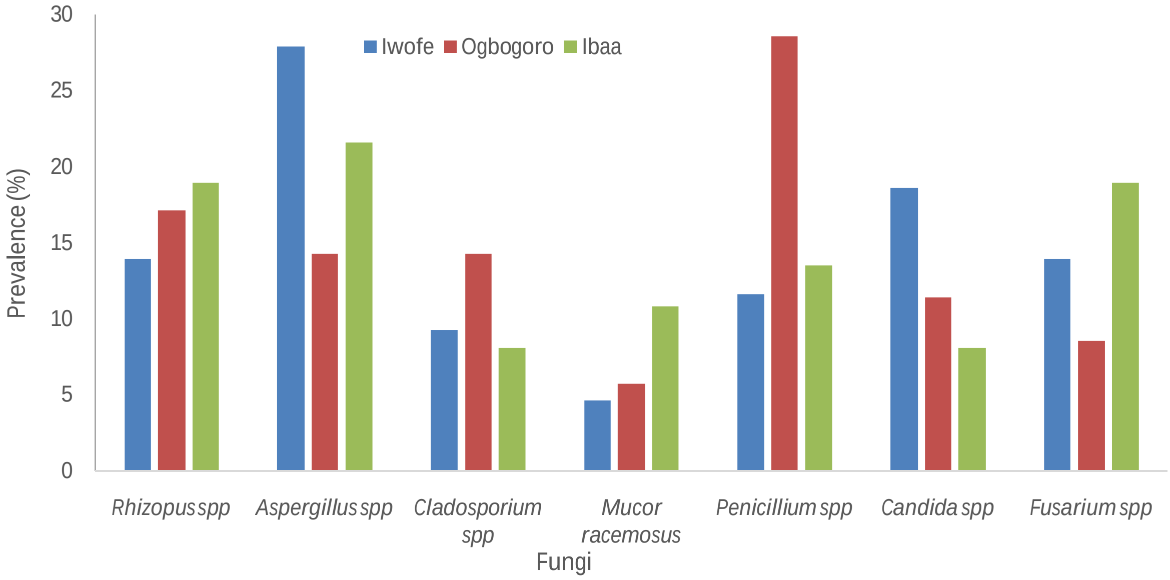

Fig.2: Prevalence of Fungi Isolated from the Artisanal Crude Oil Refining Sites

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Results of the prevalence of bacteria isolated from Ibaa, Iwofe and Ogbogorois presented in Fig. 1 and 2. The range of bacteria occurrence varies for *Pseudomonasputida* (10.9%), *Flavobacterium* species (6.9%), *Bacillus amyloliquefacius* (9.7%) and *Klebsiella*sp (4.3%). Fungal isolates from Ibaa, Iwofe and Ogbogoro percentage varies for *Pencillium* species (13.1%), *Fusarium* species (13.9%), *Aspergillus* species (21.7%) and *Mucorracemosus* (6.9%).

Discussion

Pollution of soil by crude oil and its products has been reported to impact negatively on agricultural productivity especially with microorganisms known to support plant growth (Ogbonna, 2018). The heterotrophic and hydrocarbon utilizing bacterial and fungal counts in the artisanal crude oil refining sites varied significantly. The total heterotrophic bacterial count in Ogbogorowas significantly ($p \leq 0.05$) higher than the counts recorded for Iwofe and

Ibaa artisanal refining site. The fungal counts also varied with the counts obtained from Ogbogoro and Iwofe site being significantly ($p \leq 0.05$) higher than the counts recorded in Ibaa site. The disparity in microbial counts across the locations could be attributed to factors such as the level of contaminants (crude oil residue), nutrient availability and nature of the soil. Douglas (2018) in their study also highlighted that the frequent release of crude oil and petroleum products into the soil as a result of illegal refining activities could alter the nature of the soil thereby becoming antagonistic to other soil microorganisms while supporting microorganisms that could utilize them. The total heterotrophic bacterial and fungal counts were higher than the hydrocarbon utilizing bacterial and fungal counts implying that some of the hydrocarbon utilizing microorganisms could be part of the heterotrophicmicrobial population. This agreed with Douglas and Tamunonegiyeofori, (2019) who reported similar observations. Ogbonna *et al.*(2021) reported that the toxicity of crude oil and impaired availability of nutrients to the microorganism in the contaminated soil could contribute to poor soil conditions thereby affecting the proliferation of microorganisms in the contaminated environment. A total of fifteen (15) bacterial genera and seven fungal genera were isolated from the artisanal crude oil refining sites. Out of these isolates, *Bacillus, Pseudomonas, Micrococcus, Proteus, Serratia, Aeromonas* and *Alcaligenes* sp were hydrocarbon utilizing bacteria while *Rhizopus, Aspergillus, Mucor, Penicillium, Candida* and. These bacterial and fungal isolates have been reported in previous studies by Chibuike *et al.* (2021). Chikere and Ekwuabu, (2014)also reported the presence of *Bacillus, Proteus, Klebsiella, Pseudomonas, Micrococcus, Serratia, Enterobacter, Flavobacterium, Corynebacterium* and *Azotobacter* as hydrocarbon utilizing bacteria. Bacteria genera such as *Nitrosomonas, Pseudomonas, Staphylococcus, Bacillus,Proteus* and *Citrobacter;* and fungal genera such as*Penicillium, Mucor, Rhzopus* and *Fusarium* which are similar to the isolates in the present study have been reported in a previous study (Ogbonna *et al*., 2021). They

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attributed seasonal variation as the cause of microbial diversity. The presence of co-contaminants, such as heavy metals, organic pollutants, and toxic chemicals, poses a significant challenge to the biodegradation efficiency of hydrocarbon-utilizing microorganisms. Co-contaminants can exert inhibitory effects on microbial growth and enzymatic activities, leading to a decrease in the overall bioremediation potential of microbial communities (Das and Chandran, 2011). The interactions between hydrocarbon pollutants and co-contaminants can influence the microbial community composition and functional diversity, thereby affecting the resilience and adaptability of microorganisms in contaminated environments (Hassanshahian*et al.*, 2012). The microorganisms in this study have been reported to play key roles in the utilization of crude oil. According to Douglas and Nwachukwu (2016), persistent chemical release into the environment causes a decline in biodiversity, a selective enrichment of microbial populations, and an increase in the population of organisms that can utilize or withstand these chemicals. Nonetheless, it is important to remember that the kinds and activities of microorganisms present in the soil significantly impact the soil's fertility(Douglas and Robinson, 2021). *Pseudomonas* sp and *Bacillus* sp were the most dominant bacterial isolates and their dominance could imply that the soil could support the growth of crops. Thus, why they utilize the hydrocarbons, they also serve as plant-growth promoting microorganisms. Plant growth-promoting rhizobacteria (PGPR) are beneficial bacteria that live freely and provide health benefits to crop plants. Among these isolates are the species of *Bacillus*, *Klebsiella*and *Pseudomonas* (Bashan *et al.,* 2014).

Conclusion

In conclusion, some of these organisms (bacteria and fungi) are beneficial to the environment because their presences helps to remediate the environment and detoxify harmful substance by releasing enzymes that can breakdown these constituents. Although some of the

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microorganisms in the present study have been reported to promote the growth of plant, the dumping of crude oil products legal or illegal should be minimized to avoid the extinction of plant-promoting microorganisms. Creating awareness among people living in these communitiesis highly recommended to eradicate gross pollution of the environment.

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