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Assessment of Hydrocarbonoclastic Microorganisms from Selected Mechanic Workshops in Nigeria

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ABSTRACT

Microorganisms are particularly adept at breaking down complex organic compounds into simpler, less harmful substances through processes like biodegradation and thereby offer a promising solution to the challenges posed by industrial pollutants. This study focused on the assessment of hydrocarbon-utilizing microorganisms in selected mechanic workshops within Awka Town, Anambra State and it aimed to identify microbial species capable of degrading hydrocarbons, particularly in environments impacted by automotive activities. The research methodology involved sample collection, microbial isolation, and characterization, utilizing both traditional and culture-based techniques. To achieve this, samples were collected from the workshop sites, and the isolated microorganisms were scrutinized for their hydrocarbon-degrading potential. Screening for hydrocarbonoclastic microorganisms in oil-polluted soil from mechanic workshops resulted in the isolation of bacterial, yeast, and mold strains. The identification of fast-growing *Pseudomonas aeruginosa* and *Bacillus subtilis*, along with a suspected strain of *Rhodococcus* spp., in the oil-polluted soil reinforced the report of the potential for these microorganisms in bioremediation efforts. The dominant mold was *Aspergillus* spp., known for their resilient spores suited for harsh condition, while Candida spp., was the dominant yeast genera.

1. INTRODUCTION

Bioremediation is a process that leverages the metabolic activities of microorganisms, plants, or enzymes to transform or remove pollutants from the environment. Microorganisms such as bacteria and fungi are particularly adept at breaking down complex organic compounds into simpler, less harmful substances through processes like biodegradation and mineralization, offering a promising solution to the challenges posed by industrial pollutants, hazardous wastes, and other contaminants. These microorganisms use the contaminants as energy sources through redox reactions within their cells, including respiration and other biological functions needed for cell maintenance and reproduction. Bioremediation may be conducted in situ, where the contaminated soil and groundwater are treated in place, or ex situ, where the contaminated media is removed and treated elsewhere. While traditional remediation methods exist, bioremediation stands out as a promising and environmentally conscious alternative. [1].

Hydrocarbon-degrading microorganisms are intricate microbes consisting of various microorganisms, such as bacteria, archaea, and fungi, that degrade hydrocarbon compounds. These microorganisms have evolved specialized metabolic pathways that enable them to break down complex hydrocarbons, which are organic compounds primarily composed of hydrogen and carbon atoms. The diverse members of a consortia each contribute their unique enzymatic capabilities to target different components of hydrocarbons, resulting in a comprehensive and efficient degradation process. By collaborating, these microorganisms enhance the overall degradation efficiency, particularly in environments contaminated with hydrocarbons, such as oil-polluted soils. These microorganisms play a vital role in the natural environment by helping to mitigate the environmental impact of oil spills and petroleum pollution [2].

Mechanic workshops are potential sources of environmental contamination, particularly through the release of hydrocarbons. The automotive repair process involves the

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use and handling of hydrocarbon-based substances, including oils, fuels, and lubricants. As a consequence, these workshops become hotspots for hydrocarbon contamination, with the potential to adversely impact the surrounding ecosystem.[3].

The aim of this study was to isolate and characterize hydrocarbon-degrading microorganisms from soil samples collected at mechanic workshops in Awka town, Anambra state, Nigeria. The hypothesis is that these mechanic workshop sites harbor a diverse community of hydrocarbon-degrading bacteria, fungi, and yeasts that can be harnessed for bioremediation of hydrocarbon-contaminated environments. The significance of this study lies in its potential to contribute to the development of sustainable and cost-effective bioremediation strategies for addressing environmental pollution associated with automotive activities.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in three mechanic village sites in Awka Town, Anambra state, Nigeria. Awka, the capital city of Anambra State in Nigeria, is known for its rich history as a center of blacksmithing and metalworking, dating back to the 9th century. The city is located in the savannah region of southeastern Nigeria, with a tropical climate characterized by distinct wet and dry seasons [5].

2.2 Sample Collection

Microorganisms were isolated from sand and soil samples collected at a depth of 10 cm from three hydrocarbon-contaminated mechanic workshop sites in Kwata. The samples, designated A, B, and C, were collected after removing the top soil using sterile equipment. The samples were immediately taken to the Microbiology laboratory at Nnamdi Azikiwe University for analysis. The purpose was to study the degradation of hydrocarbons using the microorganisms isolated from these contaminated sites. The three sample sites were Livinus mechanic workshop, Ifeanyi mechanic workshop, and KC mechanic workshop.

2.3 Isolation of indigenous microorganisms from the pristine soil and the oil polluted soil

Microbiological analysis of uncontaminated soil and soil contaminated by hydrocarbons was conducted using established procedures [6]. Samples, categorized as either oil-polluted or pristine (gotten from the vicinity of the mechanic workshops), were appropriately labelled. A sterile 100ml bottle containing 10 grams of contaminated soil was filled with 50ml of sterile water, shaken, and left undisturbed for 10 minutes. This step aimed to facilitate the dissolution of all particles and microorganisms from the soil before a subsequent round of shaking. One milliliter of the liquid portion from each sample was aseptically pipetted and serially diluted across five tubes. The second and fourth tubes in each set were chosen for inoculation onto Sabaraud Dextrose agar (SDA) and Nutrient agar (NA) culture plates, with the former targeting fungi and the latter targeting bacteria. Using the spread plate method, 0.1ml of each dilution (10^{-2} and 10^{-4}) from both the effluent and supernatant of uncontaminated and contaminated soil were duplicated on NA and SDA plates. Subsequently, 0.1ml of the diluted samples was added to the agar media surface and spread using a sterile curved glass spreader. The NA plates were incubated at room temperature for 24 hours, while the Sabaraud Dextrose Agar plates were incubated at 28°C for 72 hours. After incubation, colony count was done.

2.4 Isolation, purification and maintenance of pure microbial isolates

Discrete colonies from the culture plates were selected for characterization. Bacterial colonies were transferred to freshly prepared NA plates by the streak plate method and allowed to grow for 24 hours before stocking. Similarly, distinct fungal colonies (mold) were subcultured by point inoculation and streak plate method for yeast on freshly prepared Sabouraud Dextrose Agar plates for 72 hours before stocking.

2.5 Morphological Characteristics

Representative colonies of bacteria isolates were evaluated using morphological characteristics on media such as shape, colour, margin and elevation.

2.6 Biochemical identification of the isolates

The biochemical tests were performed using standard methods as previously described by [7]. The following tests were performed for the bacterial isolates: Gram stain [8], urease [8], coagulase test [8], citrate utilization [8], sugar (glucose, sucrose and fructose) fermentation tests [8], motility [9], oxidase [9], Methyl red Test [9], Voges Proskauer Test [9] and catalase [8]. Gram staining techniques for yeast cells and a lactophenol-cotton blue staining test [8] for molds were used to identify fungal isolates.

2.7 Isolation and characterization of the hydrocarbonoclastic bacteria and fungi

This was done with Mineral Salt Medium using the vapor phase transfer method as described in [10]. The mineral salt medium was sterilized by autoclaving at 121°C for 15 minutes and poured into Petri dishes. Plates were inoculated using the streak plate method from 24-hour old bacterial isolates and 48-hour old fungal isolates. Plates were inverted onto lids using 9cm Whatman No. 1 filter paper that had been previously soaked in crude oil. To suppress bacterial growth on the fungal plates, 0.5 ml of streptomycin and to suppress fungal growth on the bacterial plates, nystatin was added to the mineral salt agar. The counts of crude oil utilizing bacteria were enumerated by spread and technique using vapour phase transfer technique on Mineral Salts Agar (MSA). The number of colonies formed was used to estimate the degradation ability of the hydrocarbonoclastic bacteria and fungi [10].

$$\frac{CFU}{mL} = \frac{Average\ Number\ of\ Colonies}{Volume\ of\ Sample\ \times\ Dilution\ Value} \quad (1)$$

3. RESULTS

The total heterotrophic indigenous bacterial count was assessed from 3 mechanic workshops in Awka, revealing the highest count of 1.1×10^2 CFU/g from Sample A, followed by Sample C and Sample B with counts of 1.07×10^2 CFU/g and 6.0×10^1 CFU/g respectively. Similarly, Sample A also recorded the highest fungal count at 6.3×10^1 CFU/g, compared to Sample C which had the least fungal count of 3.4×10^1 CFU/g. Nine bacterial and six fungal isolates were identified in the soil samples collected within Awka metropolis. The tentative identity of the probable bacterial and fungal isolates considering their cultural and morphological characteristics

were, *Pseudomonas* spp, *Bacillus* spp, *Candida* spp, *Aspergillus* sp and *Rhizopus* spp.

Table 1 shows the result of the bacterial counts and fungal counts of the soil samples. Tables 2, 3 and 4 shows the colony morphology of the bacterial and fungal isolates respectively, which includes the colour, surface and shape of the isolates from the soil samples. Most of the bacterial isolates were white, creamy with a smooth surface while the fungal isolates were white, black, dark brown with a wooly or velvety surface.

Tables 5 and 6 show the biochemical characteristics of the bacterial isolates from the soil samples. Most of the isolates were catalase positive, citrate positive, coagulase negative, fructose positive and were mostly Gram positive.

Tables 7 and 8 show the biochemical characteristics of the fungal isolates from the soil samples.

Table 1. Colony counts of the isolates

Sample	Bacterial count (CFU/g)	Fungal count (CFU/g)
A1	72	67
A2a	110	63
A2b	98	61
B1	50	59
B2a	60	38
B2b	57	42
C1	94	31
C2a	107	34
C2b	102	36

Key: Sample A2: Oil-contaminated soil from Livinus mechanic workshop, Awka, Sample B2: Oil-contaminated soil from Ifeanyi mechanic workshop, Kwata, Sample C2: Oil-contaminated soil from KC mechanic workshop, Arroma

Samples A1, B1, C1 were the controls, gotten from non-polluted soils in the same environment.

Table 2. Colony morphology of the bacterial isolate

Isolates	Colour	Shape
A1	Orange	Circular
A2a	Milk	Circular
A2b	Milk	Irregular
B1	Milk	Circular
B2a	Milk	Irregular
B2b	Orange	Circular
C1	Milk	Circular
C2a	Milk	Circular
C2b	Milk	Circular

Key: Sample A2: Oil-contaminated soil from Livinus mechanic workshop, Awka, Sample B2: Oil-contaminated soil from Ifeanyi mechanic workshop, Kwata, Sample C2: Oil-contaminated soil from KC mechanic workshop, Arroma

Samples A1, B1, C1 were the controls, gotten from non-polluted soils in the same environment.

Table 3. Morphology of the Mold Growths

Isolates	Colour	Surface
A1	White	Wooly
A2	Red	Velvety
B1	Green	Velvety
B2	White	Fluffy
C1	Black	Velvety
C2	blue-green	Wooly

Key Sample A2: Oil-contaminated soil from Livinus mechanic workshop, Awka, Sample B2: Oil-contaminated soil from Ifeanyi mechanic workshop, Kwata, Sample C2: Oil-contaminated soil from KC mechanic workshop, Arroma
Samples A1, B1, C1 were the controls, gotten from non-polluted soils in the same environment.

Table 4. Colony Morphology of the Yeast Growths

Isolates	Colour	Shape	Appearance
A2	Milk	Circular	Moist
B2	Cream	Circular	Moist
C2	Cream	Circular	Moist

Key: Sample A2: Oil-contaminated soil from Livinus mechanic workshop, Awka, Sample B2: Oil-contaminated soil from Ifeanyi mechanic workshop, Kwata, Sample C2: Oil-contaminated soil from KC mechanic workshop, Arroma
Samples A1, B1, C1 were the controls, gotten from non-polluted soils in the same environment.

Table 5. Biochemical Activity of the Bacterial Isolates

Isolate	Sugar Fermentation Tests			Catalase test	Citrate test	Urease test	Oxidase test	Coagulase test	Motility test	MR-VP test
	Glucose	Fructose	Sucrose							
A1	+ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve/-ve
A2a	-ve	+ve	-ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve/-ve
A2b	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve/-ve
B1	-ve	+ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve/-ve
B2a	-ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve/-ve
B2b	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve/+ve
C1	+ve	+ve	+ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve/+ve
C2a	+ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve/-ve
C2b	-ve	+ve	-ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve/-ve

Key: Sample A2: Oil-contaminated soil from Livinus mechanic workshop, Awka, Sample B2: Oil-contaminated soil from Ifeanyi mechanic workshop, Kwata, Sample C2: Oil-contaminated soil from KC mechanic workshop, Arroma
Samples A1, B1, C1 were the controls, gotten from non-polluted soils in the same environment

Table 6. Microscopy of the bacterial isolates

Isolates	Gram reaction	Shape	Arrangement
A1	+ve	Cocci	Singles
A2a	-ve	Rod	twos, short chain
A2b	+ve	Rod	clumps, singles
B1	+ve	Cocci	Singles
B2a	+ve	Cocci	Singles
B2b	-ve	Cocci	Singles
C1	+ve	Rod	Twos
C2a	+ve	Rod	twos, short chain
C2b	-ve	Rod	singles

Key: Sample A2: Oil-contaminated soil from Livinus mechanic workshop, Awka, Sample B2: Oil-contaminated soil from Ifeanyi mechanic workshop, Kwata, Sample C2: Oil-contaminated soil from KC mechanic workshop, Arroma
Samples A1, B1, C1 were the controls, gotten from non-polluted soils in the same environment.

Table 7. Microscopy of the Yeast Isolates

Isolates	Gram reaction	Shape	Arrangement
A2	+ve	cocci	singles
B2	+ve	ovoid	singles
C2	+ve	cocci	singles

Key: Sample A2: Oil-contaminated soil from Livinus mechanic workshop, Awka, Sample B2: Oil-contaminated soil from Ifeanyi mechanic workshop, Kwata, Sample C2: Oil-contaminated soil from KC mechanic workshop, Arroma
Samples A1, B1, C1 were the controls, gotten from non-polluted soils in the same environment.

Table 8. Microscopic Structures of the Mold Isolates

Isolate	Spores present	Hyphae	Other structures
A2	Conidiospores	Septate	Stolon
B2	Sporangiospores	Aseptate	stolon and rhizoid
C2	Conidiospores	Septate	Stolon

Key: Sample A2: Oil-contaminated soil from Livinus mechanic workshop, Awka, Sample B2: Oil-contaminated soil from Ifeanyi mechanic workshop, Kwata, Sample C2: Oil-contaminated soil from KC mechanic workshop, Arroma
Samples A1, B1, C1 were the controls, gotten from non-polluted soils in the same environment.

3.1 Degradation ability of the isolates

The bacterial strains isolated during this study were tested for their ability to grow and utilize crude oil by growth on Mineral salt medium with crude oil as sole source of carbon. Rapid growth on the medium was denoted by +++ indicating high degrading ability, ++ moderate degrading ability, + low degrading ability and – no degrading ability. Four bacterial isolates showed high degrading ability, two isolates showed moderate degrading ability while three isolates showed low degrading ability. Three molds showed high degrading ability, two molds showed moderate degrading ability and one showed low degrading ability. Two yeast isolates showed high degrading ability, and one showed moderate degrading ability as illustrated in tables 9, 10 and 11.

Table 9. Hydrocarbonoclastic Abilities of the Bacterial Isolates

Isolates	Growth rate
A1	+
A2a	+++
A2b	+++
B1	+
B2a	+++
B2b	++
C1	+
C2a	++
C2b	+++

Key: Sample A2: Oil-contaminated soil from Livinus mechanic workshop, Awka, Sample B2: Oil-contaminated soil from Ifeanyi mechanic workshop, Kwata, Sample C2: Oil-contaminated soil from KC mechanic workshop, Arroma
Samples A1, B1, C1 were the controls, gotten from non-polluted soils in the same environment.

Table 10. Hydrocarbonoclastic Abilities of the Yeast Isolates

Isolates	Growth rate
A2	+++
B2	+++
C2	++

Key: Sample A2: Oil-contaminated soil from Livinus mechanic workshop, Awka, Sample B2: Oil-contaminated soil from Ifeanyi mechanic workshop, Kwata, Sample C2: Oil-contaminated soil from KC mechanic workshop, Arroma

Table 11. Hydrocarbonoclastic abilities of the mold

Isolates	Growth rate
A1	++
A2	+++
B1	+
B2	+++
C1	++
C2	+++

Key:
Sample A2: Oil-contaminated soil from Livinus mechanic workshop, Awka, Sample B2: Oil-contaminated soil from Ifeanyi mechanic workshop, Kwata, Sample C2: Oil-contaminated soil from KC mechanic workshop, Arroma
Samples A1, B1, C1 were the controls, gotten from non-polluted soils in the same environment

4. DISCUSSIONS

Based on the number of published reports, the most important hydrocarbonoclastic microorganisms in both marine and soil environments are *Achromobacter*, *Acinetobacter*, *Aeromicrobium*, *Aeromonas*, *Alcaligenes*, *Arthrobacter*, *Aspergillus*, *Bacillus*, *Brevibacterium*, *Burkholderia*, *Corynebacterium*, *Dietzia*, *Escherichia*, *Flavobacterium*, *Fusarium*, *Gordonia*, *Klebsiella*, *Micrococcus*, *Moraxella*, *Mycobacterium*, *Nocardia*, *Proteus*, *Penicillium*, *Pseudomonas*, *Rhodococcus*, *Staphylococcus*, and *Vibrio* [11].

Screening for relatively fast-growing hydrocarbonoclastic microorganisms from oil-polluted soil resulted in the recovery of four bacterial isolates, 2 yeast isolates and 3 molds. Although the isolation methods were unbiased and could select for both Gram-positive and Gram-negative bacteria, the four candidate strains were Gram-positive. *Pseudomonas* spp. emerged as the predominant bacterial genus in the screened oil-polluted soil. This finding aligns with what [12] claimed as *Pseudomonas* has been frequently associated with hydrocarbon degradation. Their metabolic adaptability makes them versatile in utilizing a range of hydrocarbons as carbon and energy sources. Their prevalence suggests a potential role in bioremediation efforts targeting oil-contaminated environments associated with automotive activities.

The fungi, *Aspergillus* spp. dominated the fungal landscape in the oil-polluted soil. Known for producing resilient spores suitable for harsh environments, *Aspergillus* species are recognized for their hydrocarbonoclastic capabilities. Their prevalence in the studied environment indicates their potential contribution to the breakdown of hydrocarbons, making them essential candidates for further investigation in bioremediation studies.

The most predominant indigenous yeast genera were *Candida* spp. While yeast genera are not often the primary focus in hydrocarbon degradation studies, the prevalence of *Candida* spp. raises intriguing possibilities. Yeasts have been known to contribute to the breakdown of hydrocarbons in various environments, and their presence in the studied mechanic workshops warrants further exploration of their potential role in hydrocarbonoclastic activities.

The best four hydrocarbonoclastic bacterial isolates displayed close morphological and biochemical similarities. Their catalase-positive, citrate-positive, and coagulase-negative characteristics align with the general traits observed in hydrocarbonoclastic bacteria according to [13]. The urease-positive nature of isolate B2a adds an interesting dimension, suggesting metabolic diversity within the selected strains.

The biodegradation of petroleum hydrocarbon can be divided into several processes according to [14]. In the first step, microorganisms enhance the bioavailability of petroleum hydrocarbon pollutants by chemotactic movements and secreting surfactants [15]. These surface-active materials increase the surface area and bioavailability of hydrophobic and water insoluble substrates, thereby increasing the speed at which petroleum hydrocarbons can approach microorganisms. Then, the petroleum hydrocarbons enter the cell through the transport process, mainly by free diffusion, passive transport, active transport, and endocytosis [16]. Finally, the petroleum hydrocarbon is degraded in the cell. The degradation pathways

of petroleum hydrocarbon compounds mainly include aerobic degradation and anaerobic degradation. Stimulated biodegradation of crude oil is currently being promoted because it ensures rapid remediation of oil-contaminated ecosystems [2]

The environmental significance of hydrocarbonoclastic organisms lies in their ability to mitigate the impact of human activities, particularly those involving hydrocarbon-based pollutants. Mechanic workshops, industrial sites, and oil spills can introduce substantial amounts of hydrocarbons into ecosystems. The presence of hydrocarbonoclastic organisms, as highlighted in studies like the one conducted in Awka South LGA, offers a natural solution for the degradation of these pollutants. Understanding the adaptive mechanisms of hydrocarbonoclastic organisms is critical for harnessing their full potential in bioremediation. Future research should focus on uncovering the specific enzymes, metabolic pathways, and genetic adaptations that allow these organisms to thrive in hydrocarbon-rich environments. Investigating the intricate interactions within microbial communities is essential. The synergistic relationships between different hydrocarbonoclastic organisms can enhance the overall efficiency of hydrocarbon degradation processes. Unraveling these microbial interactions will contribute to the development of more effective and sustainable bioremediation strategies [17].

Bioremediation plays a central role in mitigating various forms of pollution, including oil spills, chemical leaks, and industrial discharges. By harnessing the natural abilities of microorganisms and plants, bioremediation promotes the restoration of ecosystems affected by pollutants, preserving biodiversity and ecological balance. Compared to traditional remediation methods like excavation and incineration, bioremediation often proves more cost-effective. The use of natural processes and organisms reduces the need for extensive infrastructure and energy-intensive procedures. This economic advantage makes it a viable and sustainable option for environmental cleanup, especially in large-scale contamination scenarios. Bioremediation can offer long-term and sustained remediation effects. Once established, microbial populations or plants capable of breaking down contaminants can continue their activities over an extended period. This contrasts with some traditional methods that may provide short-term solutions but require continuous interventions [18].

Bioremediation aligns with environmental regulations and standards, making it an attractive option for industries seeking compliance. Governments and regulatory bodies increasingly favor sustainable and environmentally friendly remediation practices, and bioremediation fits seamlessly into this framework [19].

5. CONCLUSION

This study successfully isolated and characterized a diverse community of hydrocarbonoclastic microorganisms from soil samples collected at mechanic workshops in Awka town, Anambra state. The data obtained supports the hypothesis that these oil-polluted sites harbor a variety of bacteria, fungi, and yeasts capable of degrading hydrocarbon compounds.

The most significant finding was the isolation of four bacterial strains, two yeast strains, and three mold strains that exhibited high to moderate hydrocarbon-degrading abilities when tested on mineral salt medium with crude oil as the sole carbon source. The predominant bacterial genus was Pseudomonas, known for its versatile metabolism and adaptability to hydrocarbon-rich environments. Similarly, the fungal genus Aspergillus emerged as a prominent hydrocarbon degrader, likely owing to its ability to produce resilient spores that can thrive in harsh conditions.

While this study provides valuable insights into the native microbiome of the mechanic workshop sites, it is limited in its scope. Further research is needed to fully elucidate the metabolic pathways, enzymatic capabilities, and synergistic interactions within the hydrocarbonoclastic microbial consortia. Exploring these aspects could lead to the development of more effective bioremediation strategies tailored to the specific environmental conditions of urban mechanic workshop settings.

Overall, the data generated from this study contributes to the growing body of knowledge on microbial-driven hydrocarbon degradation. The identified strains with demonstrated hydrocarbon-degrading abilities hold promise for potential application in the bioremediation of oil-contaminated soils and groundwater associated with automotive repair facilities. Harnessing the inherent capabilities of these native microorganisms could provide a sustainable and cost-effective solution for mitigating environmental pollution in similar urban contexts.

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