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Research Article

Isolation and characterisation of hydrocarbon-degrading microorganisms from soils contaminated with used engine oil

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Abstract

Soil contamination with used engine oil is a widespread environmental issue, posing risks to ecosystems and human health. The high levels of noxious compounds, such as trace elements and organic compounds that are composed of polycyclic aromatic rings (PAHs), immediately in used engine oil can disrupt various ecosystem reformations, like biogeochemical cycling, carbon sequestration, and the degradation of humus. Soil samples were collected from mechanic workshops where there are traces of hydrocarbons, and microorganisms were isolated using enrichment techniques with used engine oil as the sole carbon source, and the isolates were subjected to some biochemical tests. The results obtained revealed that the mean count of heterotrophic bacteria ranged from 2.0 x10^8 ± 0.1 to 1.1 x 10^8 ± 0.2 CFU/g while the mean count of hydrocarbon-utilising bacteria varied from 5.4 x 10^7 ± 0.07 to 1.3 x 10^7 ± 0.05 CFU/g, and the fungal populations ranged from 2.9 x 10^7 ± 0.4 CFU/g to 1.0 x 10^7 ± 0.1 CFU/g. A total of 12 hydrocarbon-degrading bacterial species and 16 filamentous fungi were isolated, characterized and identified. Some of the characterised isolates were *Alcaligenes aquatilis* > *Pseudomonas sp.* > *Acinetobacter* >*Bacillus subtilis* > *Corynebacterium sp.*, and *Flavobacterium sp.* These oil-degrading organisms revealed hydrocarbon-degrading potential and bioremediation of the oil-contaminated sites.

Keywords: Hydrocarbonoclastic; bacteria; fungi; spent engine oil; soil contamination

1. Introduction

Terrestrial and aquatic ecosystems are becoming increasingly polluted due to the global reliance on petroleum products as a source of energy [1]. Crude oil is a liquid obtained from nature that contains a diverse range of biological molecules, primarily hydrocarbons with varying chemical and physical composition. Motor mechanics and generator mechanics frequently change motor oil and dispose of it into gutters, water drains, open vacant plots, and farmlands. This process produces spent lubricating oil, which is created when new lubricating oil is subjected to high temperature and high mechanical strain [2].

Spent engine oil poses a threat not only to human health but also to aquatic ecosystems. This is because some of the hazardous elements in wasted motor oil are long-lasting; they can build up in both terrestrial and aquatic environments and enter the food chain, where they can have catastrophic effects at higher levels [3]. As a result, used motor oil can impact fish populations through direct toxicity as well as a decline in the benthic population that serves as their food source. Due to the fact that it negatively affects soil fertility, the indiscriminate disposal of waste lubricating oil can have a significant

detrimental effect on food productivity in the terrestrial ecosystem [4]. Petroleum hydrocarbons such as aliphatic hydrocarbons, aromatic fractions, asphaltenes, polycyclic aromatic hydrocarbons (PAHs), and resins cause the main biological harm to the environment once they are present [5]. This is because they obstruct the flow of water, nutrients, oxygen, and light, which impacts soil fertility, plant growth, and germination [6].

PAHs mostly cause environmental harm when they are present in soil and sediment at concentrations above 6.5 mg/ kg and above [3, 7]. Also, PAHs have a tendency to seep into the ground after coming into contact with water. There, they remain and degrade the soil's quality and productivity, rendering it unfit for investment and agriculture [8]. Even in low quantities, PAHs can cause cancer or mutations in both humans and wildlife. Such resistant substances can be absorbed from contaminated soil via eating, breathing, or coming into contact with contaminated dust or dirt through the skin. Petrogenic hydrocarbons can build up in animal and plant tissue and spread up the food chain, killing animals and triggering genetic abnormalities in humans. This is because they linger in the ecosystem for extended periods of time [9]. Regular exposure to sub-lethal concentrations (100- 100 µg/ L) of these substances might result in a variety of physiological impairments that raise the risk of various health problems, such as hemolytic anaemia, weight loss, gastrointestinal problems, weakened immune systems, and decreased productivity [10]. In addition to having a negative impact on soil microflora and structure, aliphatic hydrocarbons can also impede the exchange of nutrients and oxygen in the soil by forming oil slicks and films [11]. Aliphatic hydrocarbons can also have an impact on the nervous system, leading to symptoms like tremors, weariness, headaches and limb numbness. Illegally discarding wasted engine oil is a global environmental risk [12]. Heavy metals found in used motor oil, such as magnesium, copper, zinc, lead, and cadmium, as well as PAHs, may be linked to long-term risks such as carcinogenicity and mutagenicity [13].

Studies on the breakdown of petrogenic hydrocarbons have demonstrated that a wide variety of microorganisms, primarily bacteria and fungi, are able to break down petroleum hydrocarbons and use them as the only carbon source for energy and metabolism, thereby remediating the polluted site [14]. Petroleum hydrocarbon compounds are broken down by a variety of microbial groups that may break down a broad range of target elements found in oil polluted ecosystem. Microbes use soluble or integral-membrane non-haem iron monooxygenases when dealing with aliphatic hydrocarbons (n-alkanes); these enzymes, known as alkane hydroxylases (e.g., AlkB), hydroxylate the substrate [15]. Fundamentally, the oxidation of the terminal methyl group, which results in the production of a primary alcohol, is typically the first step in the aerobic degradation of alkanes. Aldehyde dehydrogenases and alcohol further oxidise this product to produce the equivalent aldehyde. Finally, the resultant product is transformed into a fatty acid by oxidation. After the fatty acid and CoA couple, acetyl-CoA is transferred into the β-oxidation pathway. Both terminal and sub-terminal oxidation are used to break down long-chain alkanes [16]. When sub-terminal oxidation occurs, the secondary alcohols produced are changed to the appropriate ketone, which is subsequently oxidised by a Baeyer-Villiger monooxygenase to become an ester. An esterase is then used to hydrolyse the ester to produce an alcohol and a fatty acid.

This research explored the effectiveness of indigenous microorganisms in degrading used engine oil, contributing to practical applications.

2. Materials and Methods

2.1 Sample collection

The soil samples were randomly collected from adjoining farmlands to different automobile workshops in mechanic workshops (village) in Uyo, Akwa Ibom State, Nigeria. The soil samples were aseptically collected from ten sampling points (SPI – SP10) in to sterile plates, using spatula and conveyed to the Microbiology Laboratory, University of Uyo, Uyo, Nigeria, for analysis. At each sampling site, soil samples were collected from randomly selected points in triplicate within the used oil contaminated study area to account for spatial heterogeneity.

2.2 Microbiological analysis

2.2.1 Total heterotrophic bacterial count

Exactly 10 g of the soil contaminated with spent engine oil was suspended in 90 mL of sterile distilled water, and tenfold log dilutions (serial dilution) of the soil samples from 10^{-1} to 10^{-7} were carried out. Exactly 1 mL of the aliquot from the last dilution factor of each sample was transferred to sterile Petri dishes using the pour plate technique in triplicate for the determination of total heterotrophic bacteria. Precisely 10 mL of sterile Nutrient Agar (Oxoid) was aseptically poured, and the mixture was swirled and allowed to set on the bench. The plates were incubated at room temperature at 28 °C for 24 hours, after which observations were made for cultural characteristics.

2.2.2 Total heterotrophic fungal count (THFC)

Exactly 1 mL of the aliquot from the last diluted sample was transferred into sterile Petri dishes in triplicate. The plates were poured using Sabouruads Dextrose Agar (SDA) for the isolation of fungi. The SDA was supplemented with 0.5 mg/L of Streptomycin to inhibit the growth of bacteria. The plates were incubated at room temperature for 7 days, after which observation was made for cultural attributes.

2.3 Isolation of hydrocarbonoclastic bacteria from spent engine oil-impacted soil

2.3.1 Enrichment and isolation of bacterial isolates

The enrichment culture technique was employed. Precisely 1 g of spent engine oil impacted soil sample from mechanic village was inoculated into eight different sets of conical flasks containing 50 mL of sterile mineral salt medium [K_2HPO_4- 1.8 g, NaCl- 0.1 g, KH_2PO_4- 1.2 g, NH_4Cl- 4.0 g, $MgSO_4$- 0.2 g, $FeSO_4$- 0.001 g, per 1 liter (pH 7.0± 0.2)] enriched with 1 % of spent engine oil as carbon source. The medium was incubated at 28 ^oC in shaker incubator (100 rpm) for 7 days [17]. After 7 days of incubation, the samples were serially diluted using sterile water and plated on Nutrient Agar (NA) to obtain viable cells of bacteria. Discrete colonies obtained were sub-cultured onto fresh NA plate using streak method as described in a previous report [18] to obtain pure cultures.

2.3.2 Maintenance of pure culture of oil degrading bacterial isolates

Distinct colonies of the oil degrading bacteria isolated from the spent engine oil impacted soil was sub-cultured into McCartney bottles containing freshly prepared Nutrient Agar slants and incubated at 30±2^oC for 24 hours before storage at 4^oC for characterization.

2.4 Characterization of bacterial isolates

Oil utilizing bacterial isolates was characterized based on their cultural, morphological and standard biochemical tests as described by [19, 20]. Pure-cultures of bacteria were identified using Gram's and endospore staining as well as several biochemical test such as catalase, citrate utilization, oxidase, mannitol, Methyl red and Voges Proskauer test and sugar fermentation test.

2.5 Gram staining technique

The Gram stain is fundamental to the phenotypic characterization of bacteria and differentiation in Gram negative and Gram-positive cells. The staining procedure differentiates organisms of the domain Bacteria according to cell wall structure. Gram-positive cells have a thick peptidoglycan layer and stain violet for (purple) having picked the primary stain, Crystal violet. Gram-negative cells have a thin peptidoglycan layer and stain red or (pink), taking after the colour of the counterstain, Safranin. In a summary, the bacteria isolates were heat-fixed onto the slide by rapidly putting the slide through the flame for 20 seconds and air-dried. The fixed bacterial smear was flooded with crystal violet solution and allowed to react for one minute. Water from a slowly running tap was used to rinse off the crystal violet. After dousing it with Gram's Iodine and letting it react for a minute, it was rinsed with slowly running tap water. The smear was decoloured by flooding with 70 % alcohol and it was quickly removed (approximately one second) with the same slow-running tap. After that, preparation was counterstained with safranin and allowed for 30 seconds to react. After that, the safranin was rinse out with slowly

running tap water and Whatman No. 1 filter paper was used to blot dry. The stained smear was observed under oil immersion objective lens (100X) of the microscope. Gram-positive bacteria-stained deep violet for purple and Gram-negative bacteria stained red (or pink).

2.5.1 Citrate utilization test

The capability of the isolates to use citrate as their sole source of carbon and energy was tested using Simmon's Citrate Agar. The only carbon source in the medium was citrate, and the only nitrogen source was inorganic ammonium salts ($NH_4H_2PO_4$). Bacteria that are able to proliferate on this medium generate the enzyme citrate permease, which has the ability to convert citrate to pyruvate. The test organisms were streaked onto Simmon's citrate agar surface and cultured at 37 °C for 24 to 48 hours. A favourable response to the citrate test was indicated by colour changes surrounding the growth streak, which turned from green to brilliant blue.

2.5.2 Catalase test

This test was carried out to demonstrate the presence of the enzyme catalase that mediates the breakdown of hydrogen peroxide into oxygen and water. When a tiny inoculum is added to hydrogen peroxide, the enzyme in the bacterial isolate is clearly visible because oxygen bubbles develop quickly. The absence or ineffective generation of bubbles indicates a catalase deficiency. The inoculums must be a young culture.

Bacteria thereby protect themselves from the lethal effect of hydrogen peroxide which is accumulated as an end product of aerobic carbohydrate metabolism. Effervescence attains after 1 to 5seconds was an indicative of positive results for catalase production.

2.6 Slide method

Two drops of 3 % freshly prepared H_2O_2 were placed on a grease free slide. The test organism was transferred to the slide with the edge of sterile glass rod. Evolution of gas bubbles or effervescence of the mixture indicated positive results**.**

2.7 Oxidase examination

Oxidase enzymes play an important role in the operation of electron transport system during respiration. Cytochrome oxidase uses O_2 as an electron acceptor during the oxidation of reduced cytochrome to form water and oxidized cytochrome. A piece of filter paper was placed on a clean Petri dish. Two drops of oxidase reagent were added on the filter paper. With a sterile glass rod, the fresh test organism was smeared on the filter paper. A bright blue colouration after 1 to 5 seconds indicated a positive result.

2.8 Methyl Red and Voges Proskauer test

This test is based on the ability of some bacteria to ferment glucose with production of acid, which lower the pH of the medium to pH 4.5. A glucose phosphate broth was prepared in 1000 mL of water and autoclaved at 121 ^oC for 15 minutes; allowed to cool and inoculated with the test organism. The tubes were incubated for 48 hours at 37 ^oC.

Approximately 3 drops of methyl red were added into each tube containing 2 mL of broth culture, shaken gently and observed for colour change to bright red. Approximately 1 mL of the mixture of aqueous potassium hydroxide and 30 % alcohol alpha-naphthol was added into each test tube and shaken vigorous. Production of a pink colouration indicated a positive reaction.

2.9 Spores formation

Spore forming is one of the modes of survival for organisms during stress or unfavourable condition. Spore staining is used to identify spore forming organisms basically of the genera *Bacillus* and *Clostridium* species. A heat- fixed smear was prepared from a 24 hours old broth culture of each test organism that exhibited rod in Gram's film. The slide was placed over boiling beaker of water (steam bath) and the smear flooded with Malachite green staining solution for about 5 to 6minutes until steam

rises. Saturation was avoided by continuous flooding with the solution. The slide was allowed to cool before rinsing with water for 30 seconds. After 30 to 60 seconds of counterstaining the smear with safranin, the smear was again washed with water for 30 seconds. After using filter paper to blot dried the edge of the slide, it was examined under oil immersion objective lens (100X) of the microscope. The vegetative cells stained red while the spores stained green.

2.10 Motility

This test was used to demonstrate the ability of an organism to move with aid of motile structures called flagella. The test tube method was carried out in semi- solid medium (0.4 % NA) where a straight needle was used to touch a colony of a young (24 hours) culture growing on agar medium. Stab was done once to a depth of only one third to half inch in the middle of the tube. The test tubes were incubated at 28°C and examined daily for 3 to 5 days. Growth limited to the line of stab indicated negative results, while growth away from the line of stab indicated a motile organism.

2.11 Sugar fermentation test

This test was based on the ability of microorganisms to ferment carbohydrate by utilization of sugar as source of carbon to produce acid and gas. 1 % of each sugar was dissolved in 100 mL of peptone water. Then 10 mL of 5 % phenol red indicator already prepared separately was added to the solution and 10 mL dispensed into the test tubes with inverted Durham´s tubes. The test tubes and their contents were sterilised in the autoclave at 121 °C for 15 minutes under 15 psi. Each test organism was inoculated into each sugar tube and incubated at 37 °C for 24 to 48 hours. Tubes with yellow colouration indicated positive acid production, while tubes with yellow colouration and gas bubbles in the inverted Durham´s tubes indicated both acid and gas production.

2.12 Characterisation and identification of fungi

The fungi isolates were characterised and identified based on the taxonomic scheme reported previously [21, 22]. For characterisation of fungi, a fresh colony was placed on a clean slide and stained with lactophenol in cotton blue. It was viewed at low (X10), and high magnification (X40).

3. Results and Discussion

3.1 Total heterotrophic count in spent engine oil-impacted soil

Figure 1 shows the logarithmic values of total heterotrophic count of both bacteria and fungi in spent engine oil-impacted soil utilised for enhanced bioremediation study from different sampling points in adjoining farmlands to automobile workshops, mechanic village, Uyo, Nigeria.

The data obtained revealed bacterial densities ranged from $2.0 \times 10^8 \pm 0.1$ to $1.1 \times 10^8 \pm 0.2$ CFU/g, and hydrocarbonoclastic bacterial counts ranged from $5.4 \times 10^7 \pm 0.07$ to $1.3 \times 10^7 \pm 0.05$, while the fungal populations ranged from $2.9 \times 10^7 \pm 0.4$ to $1.0 \times 10^7 \pm 0.1$ CFU/g. It was observed that sampling point 7 (Fig. 3) exhibited the maximum mean heterotrophic bacterial count of 2.0×10^8 CFU/g, whereas sampling point 4 had the least mean (1.1×10^8 CFU/g). However, sampling point 10 (Fig. 2) had the highest mean fungal populations of 2.9×10^7 CFU/g, while sampling point 1 had the least (1.0×10^7CFU/g). The results have shown microbial assemblage and diversity in the spent engine oil-impacted soil studied.

With bacteria predominating in the microhabitats, soil-based microbes play a significant role in microbial food webs, biogeochemical cycles, and energy transport [23]. Their biodiversity can serve as a reflection of local environmental conditions because it is influenced and determined by the temporal and geographical variation of physicochemical and biological components [24]. High levels of petroleum hydrocarbons in spent engine oil decelerate the pace of biological degradation and increase the rate of biological accumulation [1]. The existence of lubricating oil in soil can have a negative impact on the natural flora abundance as well as the soil's chemical and physical characteristics.

3.2 Characterisation and identification of microbial species in spent engine oil-impacted soil

Tables 1 and 2 show the physical appearance, growth patterns and metabolic properties (morphological, cultural and biochemical characteristics) of bacterial and fungal isolates extracted from spent engine oil impacted adjoining farmland to the mechanic village in Uyo, Nigeria. The results have revealed six Gram-negative and six Gram-positive bacterial isolates. The identified bacterial species were: *Alcaligenes aquatilis, Acinetobacter* sp, *Arthrobacter* sp., *Bacillus subtilis, Corynebacterium* sp, *Flavobacterium* sp., *Miccrococcus* sp, *Pseudomonas aeruginosa, P. putida, Serratia* sp, *Sphingomonas* sp. *and Rhodococcus rhodochrous*. Fungal species were: *Amorphoteca, Neosartorya, Talaromyces, Aspergillus, Fusarium, Paecilomyces, Sporobolomyces, Cephalosporium, Penicillium, Trichoderma, Venticulum, Mucor, Candida, Absidia, Saccharomyces, Geotrichum, Phoma* and *Graphium.*

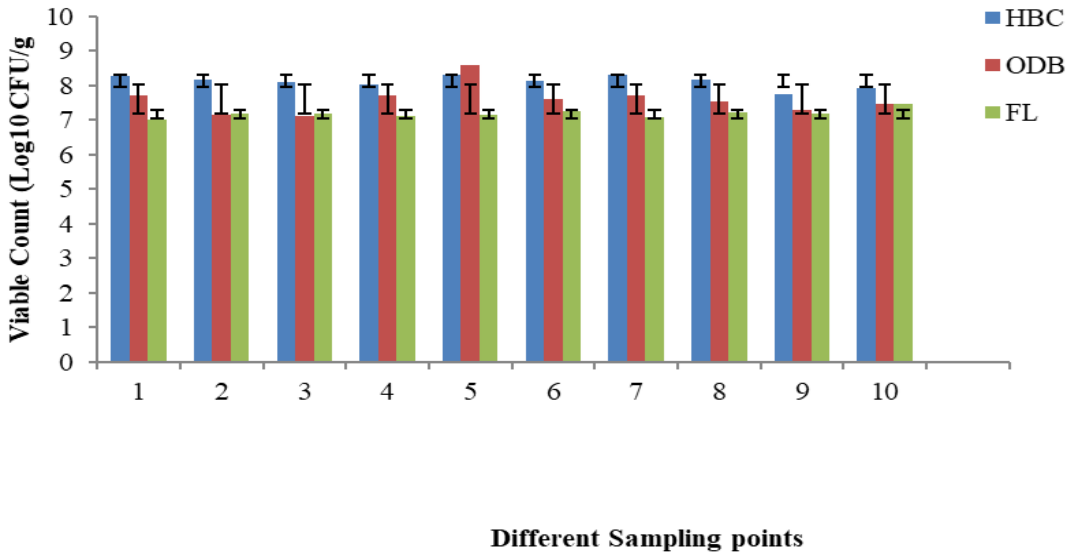

Figure 1: Total heterotrophic bacterial counts, hydrocarbonoclastic bacteria counts and fungal loads in the spent engine oil impacted soil (Keys: HBC = Heterotrophic bacterial counts; ODB= Oil degrading bacterial counts; FL= Fungal loads)

Table 1: Morphological and biochemical characteristics of bacteria isolated and identified in soil samples from the study area

S/N	Morphological characteristics				Sugar fermentation profile														Probable organism
	Colonial morphology	Pigmentation	Shape	Gram's reaction	Citrate	Catalase	Oxidase	Methyl Red	Voges Proskauer	Spore	Motility	Glucose	Mannitol	Sucrose	Lactose	Fructose	Galactose	Maltose	Mannose	
1.	Rough entire	Blue	Entire rod	-	-	+	+	-	-	-	+	-	+	-	-	+	-	-	-	*P. aeruginosa*
2.	Smooth	Blue	Circular rod	-	-	+	+	-	-	-	+	+	+	+	-	+	+	-	+	*P. putida*
3.	Rough	Milky	Irregular rod	+	-	+	-	-	+	-	-	+	-	-	-	+	+	+	+	*Corynebacterium* sp.
4.	Smooth	Milky	Circular rod	-	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	*Flavobacterium* sp.
5.	Smooth	Yellow	Circular rod	-	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	*Sphingomonas* sp.
6.	Rough	White	Irregular rod	+	-	+	-	-	-	+	+	+	+	+	-	+	-	+	+	*B. subtilis*
7.	Smooth	Milky	Regular cocci	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	*Acinetobacter* sp.
8.	Smooth	Red	Circular rod	+	+	+	-	-	+	-	+	+	+	+	+	+	+	+	+	*Serratia* sp.
9.	Smooth	Opaque	Circular rod	-	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	*A. aquatilis*
10.	Dry	Milky	Circular rod	+	-	+	+	+	-	-	-	+	-	+	-	-	+	+	-	*Micrococcus* sp.
11	Rough	Creamy	Circular rod	+	-	+	-	-	-	-	-	+	-	+	+	+	-	+	+	*R. rhodochrous*
12.	Smooth	Milky	Irregular rod	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	*Arthrobacter* sp.

Keys: + = Positive; - = Negative; sp = species

Table 2: Cultural and morphological characteristics of filamentous fungi isolated and identified in soil samples from the study area.

Code	Colony colour	Type of Soma	Nature of hyphae	Special vegetative structure	Asexual spore	Special reproductive structure	Conidial head	Vesicle shape	Probable organism
1	Glabose white	Filamentous	Septate	_	Microconidia, spindle shaped microconidia	Poorly differentiated conidiophores	_	Terminal vesicle with spine-like projection	*Microsporum sp.*
2	Smooth white	Filamentous	Septate	_	chlamydosphore	_	_	_	*Candida sp.*
3	Fluccose whitish colony with purple tinge	Filamentous	Septate	_	microconidia	Short branched conidiophores sporodochia	_	_	*Fusarium oxysporum*
4	Blue-green colony	Filamentous	Septate	Broom-like appearance	Subglobosse conidia	Highly 3-stage branched conidiophores	_	_	*Penicillum expansum*
5	White becoming grayish brown with age	Filamentous	Coenocytic	Stolons rhizoids	Ovoid sporangiospores	Tall sporangiophores in groups	_	_	*Rhizopus stolonifer*
6	Creamy white	Filamentous	Septate	Sclerotia	Conidiophore	_	Globose	_	*Aspergillus candidus*
7	Smoky or gray-green colony	Filamentous	Septate	Footcell	Globose conidia	Short conidiophores	Typically, columnar	Dome shaped broadly clavate	*Aspergillus fumigates*
8	Dense felt yellowish green colony	Filamentous	Septate	Footcell	Globose conidia	Phialides borne directly on the vesicle, scerotia	Radiate	Subglobose	*Aspergillus flavus*

Table 2 (continued)

9	Compact White or yellow basal dark colony	Filamentous	Septate	Footcell	Globose conidia	Smooth walled erect conidiophores	Globose	Globose	*Aspergillus niger*
10	Brownish colony becoming darker with age	Filamentous	Septate	Footcell	Globose conidia	Short conidiophores	Long columnor	Hemispherical	*Aspergillus terreus*
11	Grayish green	Filamentous	Septate	Footcell	Subglobose conidia	Hyaline conidiophores	Radiate	Subglobose	*Aspergillus glaucus*
12	Leathery blue green colony with red pigments	Filamentous	Septate	Broom-like appearance	Globose conidia produced in columns	1 stage branched conidiophores	-	-	*Penicillium citrinum*
13	Dense felt, gray-green spreading colony	Filamentous	Septate	Broom-like appearance	Globose conidia produced in long columns	Erect conidiophores terminating in whorl of Phialides	-	-	*Penicillium frequentans*
14	Cottony white to pale yellow	Filamentous	Septate	-	1 celled conidia in heads (cylindrical in shape)	Solitary Phialideschlamydospores absent	-	-	*Verticillium sp.*
15	White with blackish brown pycnidia	Filamentous	Septate	-	Chlamydospores, I-celled ovoid conidia	Pycnidia	-	-	*Phoma sp.*
16	Powdery olivaceous brown	Filamentous	Septate	-	Acropealbranched conidia chains	Short condiophores	-	-	*Cladosporium sp.*

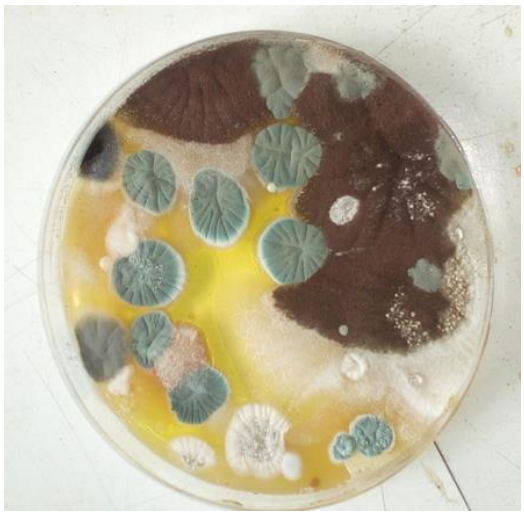

Figure 2: Sabouruads Dextrose Agar plate from sampling point 10

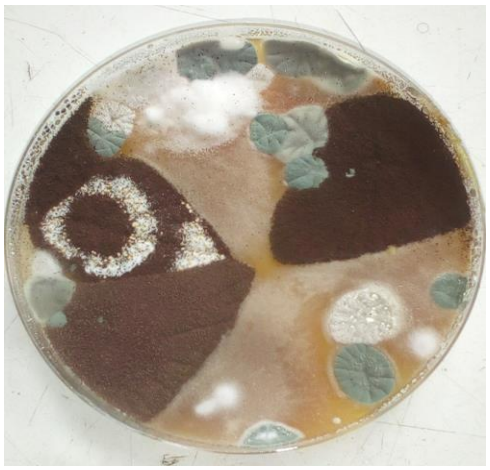

Figure 3: Sabouruads Dextrose Agar plate from sampling point 7

With bacteria predominating in the microhabitats, soil-based microbes play a significant role in microbial food webs, biogeochemical cycles, and energy transport [23]. Their biodiversity can serve as a reflection of local environmental conditions because it is influenced and determined by the temporal and geographical variation of physicochemical and biological components [25]. High levels of petroleum hydrocarbons in used engine oil decelerate the pace of biological degradation and increase the rate of biological accumulation [1]. The recovery of six Gram-negative and six Gram-positive bacteria isolates suggests adaptive selection driven by hydrocarbon stress. Petroleum hydrocarbons, heavy metals, and polycyclic aromatic hydrocarbons in spent engine oil exert selective pressure that favours organisms with catabolic versatility and membrane adaptations.

The fungal diversity observed is because filamentous fungi generally exhibit higher tolerance to harsh environmental conditions due to their mycelial growth pattern, which allows penetration into oil-polluted microhabitats and access to hydrophobic substrates. The diversity observed indicates that long-term exposure to spent engine oil has shaped a specialised microbial community capable of hydrocarbon degradation. However, while such adaptation suggests natural attenuation potential, it also reflects ecological disturbance. Ecologically, the findings confirm that used engine oil contamination significantly alters soil microbial structure, favouring oil degrading populations while potentially suppressing sensitive indigenous microbiota. Although such adaptation demonstrates resilience and

biodegradation capacity, it also signals environmental disturbance that may affect soil fertility, nutrient cycling, and agricultural productivity in adjoining farmlands.

4. Conclusion

The study provides strong evidence that soils contaminated with used engine oil in the study location serve as reservoirs of functionally competent hydrocarbon-degrading microorganisms. These isolates represent valuable candidates for biotechnological applications, particularly in the development of cost-effective and environmentally sustainable bioremediation strategies for petroleum-polluted environments. Future research should focus on molecular characterisation, gene expression profiling of degradation pathways, and pilot-scale bioremediation trials to fully harness the biodegradative potential of these indigenous microbial communities.

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Conflict of Interests
The authors declare that they have no conflict of interest.

Author Contributions
All authors have read and approved the final version of the manuscript.

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