



Utilization of Decane, Heptane, Petrol and Hexadecane by Fungi Isolated from Engine Oil Contaminated Soil in Ilorin

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ABSTRACT: Fungi with hydrocarbon degrading capabilities were isolated from spent engine oil contaminated soil obtained from five automobile workshops in Ilorin, Nigeria. Preliminary analysis of degradation of the hydrocarbon was performed using Mineral Salts Medium (MSM). The organisms identified include *Aspergillus* sp., *Blastomyces dermatitidis*, *Cladophialophora bantiana*, *Mucor circinelloides*, *Phaeoacremonium parasiticum*, *Rhizopus arrhizus*, *Neosartorya fischeri*, *Cladosporium* sp. and *Microsporium furrigenum*. They were identified based on their morphological and cultural characteristics. Findings from this study showed *Aspergillus* sp. and *Mucor circinelloides* were able to degrade hexadecane, heptane, decane and petrol as sole carbon and energy source within 20days. Hydrocarbon utilization rate ranged from 0.020 to 0.284 for heptane, 0.0120 to 0.4965 for decane, 0.079 to 0.340 for hexadecane and 0.023 to 0.268 for petrol. Measurement of optical density and pH was carried to determine hydrocarbon degradation by fungi. The results of this study shows that fungi can play important roles in the effective clean-up of hydrocarbon contaminated soils thereby making them environmentally friendly.

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Development and technological advancements have characterized the production, distribution and use of petroleum products. Anthropogenic activities such as industrial and municipal effluent discharge, activities of offshore and onshore petroleum industries as well as accidental spills cause pollution (Sajna *et al.*, 2015). Petroleum products like diesel, engine oil, kerosene and petrol are used daily in automobile workshops. Most auto-mechanic workshops in many developing countries such as Nigeria lack regulations and standards to guide their operations and disposal of used engine oil and other petroleum products (Kpakpavi, 2015). The careless disposal of these products results in pollution of the soil and aquifer (Onifade *et al.*, 2007) and pollution of the soil by hydrocarbons is hazardous to the ecosystem as it results in depletion of plant nutrients, bioaccumulation of pollutants in plants as well as bioaccumulation and biomagnification in animal tissues thus; leading to acute or chronic health effects, mutations and consequently, death (Ekhaise and Nkwelle, 2011; Oluwajiose *et al.*, 2105; Nkwoada *et al.*, 2018). Globally, the spills from crude oil and removal of hydrocarbons from the environment have posed a great challenge, but the use of microorganisms in bioremediation of polluted sites has become an efficient technique to ameliorate pollution (Karthika *et*

al., 2017). Omotayo *et al.* (2012) established that the success of oil spill clean-up is dependent on the presence of microorganisms with appropriate metabolic abilities. However, factors such as adsorption rates and low solubility have restricted the utilization rates of hydrocarbons by microorganisms (Millioli *et al.*, 2009). Soil is a major component of the ecosystem and also an important carbon sink in the environment (Davidson *et al.*, 2006). Soil is the habitat for variety of organisms, including fungi, bacteria, protozoa, viruses, insects, nematodes, worms, and many other animals. These complex biological communities contribute to the formation, maintenance, and in some situations, the degradation and disappearance of soils (Prescott *et al.*, 2005). Microorganisms such as fungi are extremely diverse and can adapt to survive in harsh environments and are capable of breaking down many complex molecules by adaptation of their degradative enzyme system (Boonchan *et al.*, 2000). Fungi are abundantly found in a host of environments, and are independent, parasitic or mutually beneficial in existence. Fungi are vital in maintaining the proper functioning of the ecosystem by decaying dead matter and releasing vital nutrients in the soil and atmosphere (Natasha, 2019). They are producers of extracellular enzymes that make them capable of metabolizing pollutants. These

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contaminated sites are a reliable niche for the assimilation of substrates which they utilize as carbon source (Ojo, 2006). Many studies have shown the role of fungi in the biodegradation of various petroleum products and the most common biodegraders are of the genera: *Aspergillus*, *Candida*, *Mucor*, *Penicillium*, *Geotrichum*, *Alternaria*, *Rhizopus* etc (Husaini *et al.*, 2008). This work aims to establish the ability of fungi isolated from soil contaminated with spent engine oil to utilize hydrocarbons.

MATERIALS AND METHODS

Collection of Soil Samples: Samples contaminated with spent engine oil were collected from five (5) automobile workshops located in Tanke, Ilorin. The samples were collected in the morning before the workers get to their workshop.

Sample Bioassay: Preparation of media was carried out in accordance with the directions provided by the manufacturer. All media along with Petri dishes, pipettes, glassware were sterilized in an autoclave at 121°C for 15 minutes. Pouring of media into plates as well as other microbiological processes was carried out aseptically. For preliminary study of engine oil degrading fungi, time course degradation of the hydrocarbon was performed using mineral salts agar medium described by Vecchioli *et al.*, (1990). The composition of the mineral salts medium used was MgSO₄.7H₂O (0.2g), KNO₃ (0.3g), KH₂PO₄ (0.5g), Na₂HPO₄ (1.4g), (NH₄)₂SO₄ (1.0g), Distilled water (1L). The MSM bottles were inoculated in 36 bottles with 18 controls: 12 bottles for each hydrocarbon and 6 controls for each hydrocarbon. The chemicals were measured using aluminum foil on a weighing balance and dispensed into an Erlenmeyer flask and 250ml of water was poured into the flask and swirled till completely dissolved. It is then poured into a washed 1litre keg and 70ml of water is added and shaken. 20ml is dispensed into each falcon's bottle and sterilized by autoclaving at 121°C for 15mins and wait to cool.

Isolation of Fungi from Diesel Contaminated Soil: Exactly 5 grams of Saboraud Dextrose Agar (SDA) was measured on a weighing balance and dispensed into an Erlenmeyer flask and 125g of water was dispensed into it and swirled till no visible lumps. It was filtered into a falcon's bottle, covered and sterilized by autoclaving at 121°C for 15mins. The enumeration of fungi was done using serial dilution plating method. 1g of soil samples from each site was dispensed in a 9ml sterile distilled water and labeled respectively. It was then diluted tenfold up to 10⁻⁵. Plating was done at a serial dilution of 10⁻⁵ and 10⁻⁴ respectively. Streptomycin was added into the media to inhibit bacteria growth, poured into the Petri dish

and swirled. Incubation was carried out at room temperature for three (3) days.

Characterization and Identification of Fungal Isolates: The fungi that grew on the media were considered as degraders of hydrocarbons. After the third (3) day, they were aseptically smeared on a glass slide, stained with lactophenol blue and covered with a cover slip and examined accordingly. The isolates were identified according to their macroscopic and microscopic characteristics based on their form, elevation, margin, size, colour, opacity and surface as reported by (Aneja, 2007; Fawole and Oso, 2007).

Evaluation of the ability of fungi to degrade hydrocarbon - heptane, decane, hexadecane and petrol: Measurement of Optical Density: Mineral Salts Medium (MSM) prepared for the fungal isolates contained 0.5g/l KH₂PO₄, 1.4g/l Na₂HPO₄, 0.2g/l MgSO₄.7H₂O, 0.3g/l KNO₃, 1.0g/l (NH₄)₂SO₄. 20ml of the Mineral Salts Broth (MSB) was measured inside 16 reaction bottles. 12 of the reaction bottles were divided into 2 for day 1,4,8,12,16 and 20 respectively while the remaining 4 reaction bottles were used as control. All the reaction bottles containing the Mineral Salts Broth were sterilized in an autoclave at 121°C for 15 minutes. 26µl of hexadecane was measured inside 20ml of MSB and the measurement of heptane was gotten from this calculation according to Zampolli *et al.* (2014): The micropipette was set to measure 26µl of hexadecane and was put into sterile Mineral Salts Medium (MSM). The fungi isolate was added into the 20ml mineral salts medium and incubated for three days. The plates were then incubated at 25°C. The optical density (OD) was monitored and recorded every four (4) days for twenty (20) days. The measurement of OD was done using a spectrophotometer at a wavelength 600nm according to (Kumar and Manjunath, 2015). This procedure was repeated for heptane (33µl), decane (27µl) and petrol (26µl) all of which were added to 20ml of Mineral Salts Medium respectively.

Measurement of pH: Growth of *Aspergillus* sp. on spent engine oil substrate was carried out by the inoculation of each of the fungal cultures into a 250ml Erlenmeyer flask as described by Oboh *et al.* (2006). The isolate was added and left for 3 days. 1.5ml of the isolate was supplemented into each falcon bottle as a carbon source. The falcon tubes were tightly closed and incubated at room temperature and readings taken at an interval of 4 days for 20 days. The pH meter used was calibrated with buffer of pH 7.0 before use and readings from the pH meter were noted and recorded accordingly.

RESULTS AND DISCUSSION

A total of sixteen (16) fungi were isolated in this study and they were identified based on their morphological and cultural characteristics. However, nine (9) of the fungi were identified due to their appearances on different plates. The organisms identified include *Aspergillus* sp., *Blastomyces dermatitidis*, *Cladophialophora bantiana*, *Mucor circinelloides*, *Phaeoacremonium parasiticum*, *Rhizopus arrhizus*, *Neosartorya fischeri*, *Cladosporium* sp. and *Microsporium furrigenum* which agrees with those

reported in the researches of Obire and Anyanwu, (2009) and Romeo *et al.* (2010). Fungi are capable of biodegradation due to their resistance to hydrocarbons. An example of a good fungal biodegrader is *Aspergillus* sp. which is capable of degrading a wide range of hydrocarbon pollutants. The isolation of these fungi from the contaminated sites may be as a result of their ability to synthesize enzymes that enable them to utilize the spent engine oil in soil thereby causing (Zhang *et al.*, 2016a). Table 1 gives the characteristics of the fungal isolates that could degrade the spent engine oil and most of them were found to be molds.

Table 1: Characterization of fungal isolates

Cultural Characteristics	Microscopic Characteristics	Probable Isolates
White/cream colour non-sporulating colonies	Large, yeast forms, single bud attached by a broad base	<i>Blastomyces dermatitidis</i>
Black to green colour olive to purple-brown reverse side	Conidia heads are short, columnar, Hyphae was small with distinct cross-septa	<i>Aspergillus</i> sp.
White to pale yellow colour colony with good growth	Conidia heads are short, columnar	<i>Neosartorya fischeri</i>
Moderate growth colony, grey-suede to floccose colour	Conidia sparsely branched and very long	<i>Cladophialophora bantiana</i>
Waxy surface colony and slow growing	Irregular branching hyphae with prominent cross walls	<i>Microsporium furrigenum</i>
Brown/grey, floccose slow growing colony	Vegetative hyphae, Conidiospores and conidia pigmented	<i>Cladosporium</i> sp
Whitish-grey colony	Conidia, phialide are funnel-shaped	<i>Phaeoacremonium parasiticum</i>
White cotton to brownish grey blackish-grey color	Non-septate sporangiospores	<i>Rhizopus arrhizus</i>
Floccose, pale greyish-brown colour with poor growth	Branched sporangiospores bearing brown-like sporangia	<i>Mucor circinelloides</i>

The degradation of petroleum and its derivatives depend on the concentration and composition of the petroleum hydrocarbon (PAH), abiotic factors as well as microbial biomass in the soil. These conditions make microorganisms respond to pollution (Atagana *et al.*, 2006). Fungi are not dependent on soluble, organic compounds for nutrition hence; they are capable of secreting different enzymes that get attached to polymeric substances (Harms *et al.*, 2011). The work of Uzoamaka *et al.* (2009) authenticated that fungi isolates obtained from oil contaminated soils had biodegradation abilities. To correlate this, hydrocarbon utilizing fungi which included *Aspergillus fumigatus*, *Aspergillus niger*, *Penicillium xingjiangense*, *Mucor racemosus* were isolated from polluted soils in the study of Edna *et al.* (2016) however, *Aspergillus* sp. and *Penicillium xingjiangense* were found to be more effective in hydrocarbon degradation. Figure 1 is descriptive of the occurrences of fungi isolates at the different sample sites.

pH and Optical density determination: Soil pH is crucial for enzymatic activities. The pH of soil affects substrate availability. Extreme pH can denature enzymatic structure and prevent the enzyme from

acting on available substrates in the soil (Zhang *et al.*, 2016b). Figure 2 shows the pH values of the Mineral Salts Medium in the engine oil degraded samples. From the chart, the highest pH value obtained was 6.85 while the lowest was 5.62 and this shows that hydrocarbons are substances that partially dissociates into ions when dissolved in a solvent (i.e. they are weak acids) as stated by Jönsson *et al.* (2013).

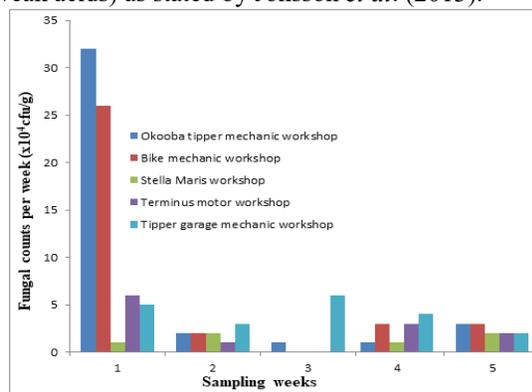


Fig 1: Total fungal counts

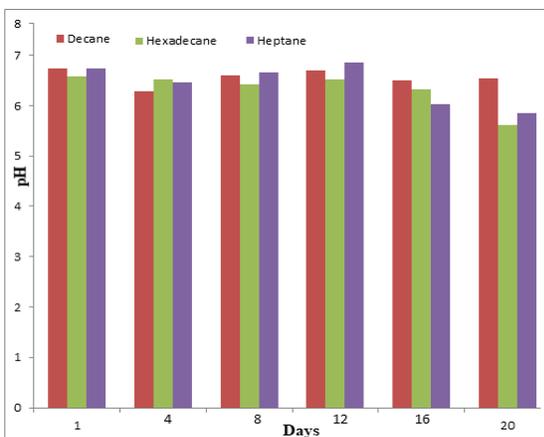


Fig 2: pH values of the Mineral Salts Medium in the engine oil degraded samples.

In Figure 3, growth configuration showed that there was slight significant difference in microbial growth of *Aspergillus niger* as evidenced in the decline on the growth curve for Day 2. Bagherzadeh-Namazi *et al.* (2008) reported that peak reduction is a strong evidence for degradation. The slow degradation showed from Day 2 to Day 4 by the optical density readings is probably due to climatic conditions, temperature and nutrient availability which are the major factors affecting biodegradation (Xiaoxu and Joel, 2019). Utilization of hexadecane, heptane, decane and protein by *Mucor circinelloides* in Figure 4 shows a higher nutrient uptake. The figure shows a value decrease on Day 5 and peak increase on Day 6 for petrol, decane and heptanes respectively meaning that *Mucor circinelloides* continued to utilize the hydrocarbon present in the mineral salts medium depicting availability of nutrients. Generally, petroleum hydrocarbons in soil affects moisture holding capacity, soil permeability, microbial growth, physicochemical properties of soil and all these ultimately sum up in the damage of the environment (Ezeigbo *et al.*, 2013; Zhang *et al.*, 2018).

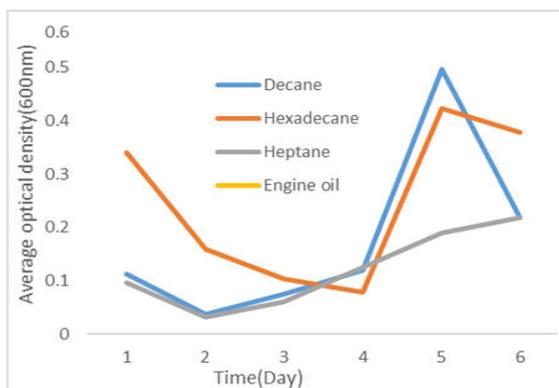


Fig 3: Optical density measurement of *Aspergillus sp.*

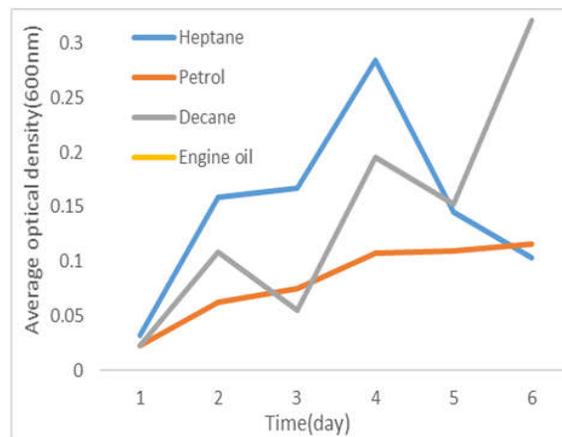


Fig 4: Optical density measurement of *Mucor circinelloides*

Microbial degradation of crude oil is often shown to occur by attack on alkanes or light aromatic fractions, while the higher molecular weight aromatics, resins and asphaltenes are considered recalcitrant. Crude oil, particularly the aromatic fraction is acutely lethal to agricultural soils at varying concentrations. Affected soils lose their fertility and become unproductive but within a few years, crude oil contaminated soils return to full productivity when appropriate clean-up techniques are used (Kisic *et al.*, 2009; Parlak and Parlak, 2011).

Conclusion: Contamination of the soil by petroleum products alter soil properties and result in a significant level of toxicity in the soil. Thus, use of microorganisms as bioremedial tools has become a highly recognized technique in tackling environmental pollution caused by oil spills hence; this study buttresses the exploration and utilization of fungi species for the efficient bioremediation of petroleum and its derivatives in the environment.

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