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Investigation of Fungal Degradation Efficiency on Spent Engine Oil

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ABSTRACT

The contamination of soil by spent engine oil is on the increase due to increased use of engines that use petroleum products. This study was aimed to investigate the biodegradation of spent engine oil using indigenous fungi isolated from soil samples. Engine oil contaminated soil was collected from two mechanic workshops located around Alex Ekwueme Federal University Ndufu-Alike. From the collected samples indigenous fungi were isolated using Sabouraud Dextrose Agar. A total of thirty isolates (100%) were isolated among which *Aspergillus sp* (60%) and *Penicillium sp* (30%) were found to be predominant. Morphological studies of the fungal isolates showed different growth patterns. Screening of the isolates for biodegradation potential was done using the Minimal Salt Medium, where six (20%) of the fungal isolates that passed the screening test were used. Biodegradation study was conducted for 15 days, isolates of *Aspergillus* CA4(2), *Penicillium* CB3(1), and *Penicillium* CA2(2) had a percentage rate of degradation of 78.9%, 72.1% and 65.9% respectively, while the isolate of *Aspergillus* CB3(3) recorded the least at 34.9%. The GC-MS result showed loss of hydrocarbons compound from the residual degraded oil and a 100% removal of pentadecane. The result revealed that the fungal isolates indigenous to contaminated engine oil sites have the potential to breakdown hydrocarbons, which makes them potent organisms for environmental cleanup.

Key words: Investigation, Fungi, Engine oil, Minimal Salt Broth, Aspergillus

INTRODUCTION

Pollution from petroleum and its derivatives has become a global problem, especially in developing countries like Nigeria. The improper disposal of used engine oil by mechanics during oil changes is common, with oil frequently dumped into runoffs, gutters, vacant lots, and farmland. This oil often remains untreated for years, leading to soil and water contamination that poses serious environmental risks. The presence of spent oil in the soil creates unfavorable conditions for plant growth, including poor aeration, nutrient immobilization, reduced water retention, lower pH, and decreased enzyme activity (Adongbede & Sanni, 2014). Used engine oil differs from unused oil in that it contains additional harmful chemicals accumulated during engine operation. The presence of petroleum hydrocarbons is particularly concerning, as many polycyclic aromatic hydrocarbons (PAHs) are toxic, mutagenic, and carcinogenic (Fulekar *et al*., 2013). Used motor oil also contains metals such as aluminum, chromium, copper, iron, lead, manganese, nickel, silicon, and tin, which can lead to various health issues, including mutations and cancer. Plants exposed to contaminated soil may suffer from chlorosis, leaf bleaching, necrosis, malformations, reduced yield, and impaired fertility.

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In recent years, fungi have gained attention for their potential in bioremediation, capable of degrading various materials through myco-degradation (Barnes *et al*., 2018). Fungi are essential to soil ecosystems, contributing to nutrient cycling and biotic interactions through their mycelial networks (Ritz, 2004). Their advanced enzymatic systems enable them to thrive on a wide range of natural and synthetic substrates, breaking down complex compounds into smaller, absorbable molecules (Sardrood *et al*., 2013). The mycelial structure enhances their ability to bioremediate extensive contaminated areas due to its large surface area (Bennet *et al.,* 2002). Numerous studies have investigated the ability of native fungal strains to degrade petroleum hydrocarbons (Maddela *et al.,* 2016; Victor-Ekwebelem *et al.,* 2020). These strains are better adapted to local soil conditions, making them more competitive against other organisms. Specifically, saprotrophic strains that decompose complex organic matter like lignin show promise for bioremediation (Godoy *et al.,* 2016). Isolating cultivable fungi from oil-polluted soils yields strains better suited to contaminated environments than those from culture collections (Godoy *et al.,* 2016). Therefore, this study aims to biodegrade spent engine oil using indigenous fungi isolated from oil-polluted soil samples.

MATERIALS AND METHODS

Study Area

The study was conducted on soil samples collected from mechanic workshop located around Alex Ekwueme Federal University Ndufu-Alike in Ikwo local government area Ebonyi state. The majority population of the people in this area are farmers and engage in petty trade and business. Ebonyi state is a state in the Southeastern part of Nigeria. Its geographical coordinates are a longitude of 8°06'E and a latitude of 6°20'N with a tropical climate, i.e., rainy season (April to October) and dry season (November to March). Its vegetation is the sub-savannah rainforest. The state's estimated population was 2.2 million in the 2006 census (National Bureau of Statistics, 2012).

Study Design

A cross-sectional study was conducted to characterize indigenous fungi that has the capacity to biodegrade used engine oil isolated from contaminated soil. Engine oil contaminated soil was collected randomly from mechanic workshop at AE- FUNAI area and Junction area of Ikwo local government following aseptic conditions.

Sample Collection

Two soil samples were collected using a soil auger from the top soil surface to a depth of 30cm deep by digging. Both samples were labeled with a code CA and CB. The soils were transferred into a sterile polythene bag and then transported to Microbiology laboratory at AE-FUNAI for further analysis.

Isolation of Fungi Isolates

Normal saline was prepared using 0.85g of sodium chloride in 1000ml of distilled water and was dissolved in a conical flask and sterilized using the autoclave for 15mins at 121^{o}C, 9mL of normal saline was poured into 9 different test tubes and allowed to cool off. Ten grams (10g) of the soil sample labelled sample CA was weighed using the weighing balance and transferred into beaker containing 10mL of distilled water, it was mixed thoroughly for 30mins until a homogenous mix was obtained, then 1mL was taken from the mixture and transferred into the first test tube containing 9mL of distilled water, thereafter a two-fold serial dilution was done from 10^{1} to $10^{10.}$ Similarly, same process as above was

done on soil Sample labelled CB. After which 0.5mL of the diluent was taken from test tube label 10^6 to 10^{10} into a molten sterile Sabouraud Dextrose Agar (SDA) infused with 2g of ciprofloxacin using pour plate method, the plates were swirled to have a homogenous mixture. This process was repeated for samples CB. The agar was then left to solidify. Thereafter the plates were then incubated for 48hrs to 72hours at 25°C. After incubation readings were taken by counting the number of colonies seen on each plate.

Purification and Maintenance of Pure Cultures

The colonies that developed on the SDA agar plate was repeatedly subculture to obtain pure culture. To maintain pure culture of the isolates, SDA was prepared according to manufacturer instruction and sterilized at 121°C for 15 minutes, after which it was poured into bijou bottles autoclaved and allowed to solidify in an angle of 45°. Distinct colonies from the pure culture of each of the plates prepared for both sample CA and CB were inoculated into the slant bottle following aseptic condition and then incubate at 25°C for 48 hours and were stored at 4°C for further use.

Screening of Fungal Isolates for Biodegradation Ability

All pure fungal isolates were screened for their ability to utilize the spent engine oil as the carbon source. Minimal salt medium containing 0.05g of KH_2PO_4, $(NH_4)2SO_4$ 0.05g and 0.05g of $MgSO_4.H_2O$ in 1000mL was prepared, then 10mL was transfer into test tubes and 5mL of the spent engine oil was added into each of the test tube before been sterilized in the autoclave for 15minutes according to Udeme *et al*. (2023). After sterilization the test tubes were allow to cool, after which 1mL of isolate was inoculated into each of the test tubes under aseptic conditions. The test tubes were labelled based on the isolates and control was also made available. The control contains only the sterilized media and the oil without fungal isolates whereas the treatment group contain the media, oil and isolates. Both the control and treatment group were incubated at 25°C for 48 hours. The tubes were observed for turbidity visually and level of the oil was noted. Test tubes that were turbid with reduced oil level were selected for further analysis.

Characterization and Identification of the Isolates

Identification of fungal isolates

The colonies on the plates were identified macroscopically and microscopically. Colony color, type, texture shape and growth pattern were observed. The fungal isolates were stained with lactophenol cotton blue dye and was the stained slide was viewed under the light microscope (at x10 magnification). Identification was done using fungi atlas manual and guides according to Watanabe (2002).

Biodegradation Studies

A total of 12 (40%) fungal isolates out of 30 were selected based on the screening result and subjected for Biodegradation studies.

Preparation of isolates for biodegradation set-up

Nutrient Broth was prepared according to the manufacturer's direction and 5mL of Nutrient Broth was dispensed into 13 test tubes, the test tubes were sterilized in an autoclave and was allowed to cool completely. Then each pure isolates were inoculated into the cool nutrient broth and was well labelled. All test tubes were incubated in an incubator at 37°C for 18hours.

MSM (Mineral Salt Media) preparation

Mineral Salt Medium was prepared using: Dipotassium hydrogen phosphate of 1.72g, 0.68g of Potassium dihydrogen phosphate, 0.1g of Magnesium sulphate heptahydrate, 4g of

Sodium chloride, 0.03g of Ferrous sulphate heptahydrate, 1g of Ammonium nitrate and 0.02g of Calcium chloride in 1000mL of distilled water. It was allowed to properly dissolved then One hundred milliliter (100mL) of Mineral Salt Medium was dispensed into each Bioremediation bottles and weighed. Two milliliters (2mL) of spent engine oil were added into each bottle, the mixture was sterilized. Thereafter Two milliliter (2mL) of each organism were added to the mixture containing crude oil and MSM, all content were weighed and kept in a safe place where it was aerated intermittently for two weeks. Measurements were taken every two days interval for two weeks to measure the rate of biodegradation.

Extraction of residual oil

Twenty-five milliliters (25mL) of diethyl ether were poured into the bioremediation bottles containing the mixture, then shaken thoroughly. The mixture was poured into a separating funnel attached to a retort stand and allowed to separate the water from the oil, two layers were formed; the water layer and the oil layer, the water layer was allowed to flow out leaving behind the oil layer. This was collected using the universal bottle with known weight. They were kept in a safe place for some days leaving them open to allow it to evaporate. After evaporation the weight of the residual crude oil was taken and recorded.

The amount of degraded crude oil was calculated using the formula below (Udeme *et al.,* 2023):

Weight of residual oil = Weight of container and oil – Empty container

Crude oil degraded = Original weight of crude oil – residual oil

$$\% \text{ degradation} = \frac{\text{Amount of oil degraded}}{original\ oil} \times 100$$

RESULTS

Count of Bacterial Isolate for Sample CA and CB

The enumeration of fungal isolates from sample CA and CB on Sabouraud Dextrose Agar. The total colony count of Sample CA1 recorded the highest of 7.4×10^{-4} Cfu/g and CA5 recorded the least for 2.2×10^{-9} Cfu/g. On the other hand for sample CB, CB1 recorded the highest of 8.0×10^{-5} Cfu/g and CB5 recorded the least for 9.0×10^{-9} Cfu/g (Table 1).

Table 1: Count of bacterial isolate for sample CA And CB

Sample code	Volume of diluent (ml)	Dilution factor	CFU/g	Sample code	Volume of diluent (ml)	Dilution factor	CFU/g
CA1	1.0	10^{-6}	7.4×10^{-4}	**CB1**	1.0	10^{-6}	8.0×10^{-5}
CA2	1.0	10^{-7}	5.9×10^{-5}	**CB2**	1.0	10^{-7}	2.5×10^{-5}
CA3	1.0	10^{-8}	4.8×10^{-6}	**CB3**	1.0	10^{-8}	1.8×10^{-6}
CA4	1.0	10^{-9}	4.1×10^{-7}	**CB4**	1.0	10^{-9}	3.8×10^{-7}
CA5	1.0	10^{-10}	2.2×10^{-9}	**CB5**	1.0	10^{-10}	9.0×10^{-9}

Key: CA = Sample code, CB = Sample code.

Screening Result of the Selected Fungi Isolates

Out of the twelve (12) fungal isolates screened, 6(50%) had high degradation efficiency designated with ++, as they were able to grow and reduce the oil level in the MSM containing Spent engine oil as the carbon source. Three 3(25%) of the isolates were intermediate while isolates CB2 (2), CB1 (3) and CA2(3) had no visible growth in the media and was recorded as negative (-) (Table 2).

Table 2: Screening of fungal isolates for degradation ability

Isolate Code	Result	Isolate Code	Result
CA3 (2)	++	CB3 (1)	+
CB4 (3)	++	CA3 (2)	++
CB3 (1)	++	CB1 (3)	-
CB3 (3)	++	CA2 (2)	++
CB5 (2)	+	CA2 (3)	-
CB2 (2)	_	CA2 (1)	+

Key: ++- Oil level reduced; isolate grows (medium becomes turbid). + - Isolate grows only without oil level not been reduced.

Characterization of Isolated Fungi Isolate

The cultural characteristics, the shape, color, texture and elevation of each colony were observed. The major fungi identified as correspond to fungi atlas were *Aspergillus* spp. and *Penicillium* spp (Table 3).

Table 3: Characterization of the fungal isolates used for the biodegradation study

Test	Fungi isolates					
Carbohydrate Fermentation	**CA3(2)**	**CB4(3)**	**CB3(1)**	**CB3(3)**	**CA2(2)**	**CA4(2)**
Starch hydrolysis	+	+	+	+	+	+
Glucose	+	+	+	+	+	+
Galactose	-	-	+	-	+	-
Fructose	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+
Lactose	-	-	-	-	-	-
Cultural xtics	Oval in shape, present of characteristics bud, and irregular branched hyphae	Oval in shape, present of characteristics bud, and irregular branched hyphae.	The hyphae are generally septate. Slender and branched and spherical in shape.	Oval in shape, present of characteristics bud, and irregular branched hyphae.	The hyphae are generally septate. Slender and branched and spherical in shape.	Oval in shape, present of characteristics bud, and irregular branched hyphae.
Probable Fungi	*Aspergillus spp.*	*Aspergillus spp.*	*Penicillium spp.*	*Aspergillus spp.*	*Penicillium spp.*	*Aspergillus spp.*

Percentage Rate of Degradation

There was a progressive decrease in the weight of the oil and the rate of degradation was observed to be (2.0 %) for day 1 and day 3 (4.9 %) even due it was not significant at $p \geq 0.05$, change in amount of spent oil and rate of degradation were significant at $p \geq 0.05$, at day 5(14.6%) and day 7 (23.5%), for *Aspergillus* CA4(2) the weight of oil progressively decreased from 3.17g on day 0 to 3.12 (day 1), 2.72 (day 5) and 2.52 (day7) with a corresponding rate of degradation of 0%, 1.5%, 14.20% and 20.51% for day 0, day 1, day 5 and day 7 respectively (Figure 1a), while the controls remained constant (Table 4). Similarly the decrease in weight of oil and increase in the rate of degradation continued progressively from day 9 to day 15 in the order 63%, 65% and 69.27% for *Penicillium* CB3(1) (Figure 1b). the controls remained constant (Table 4).

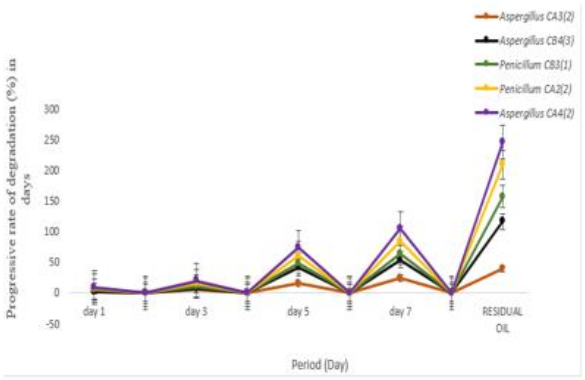

Figure 1a: Progressive rate of degradation of spent engine oil after 7 days

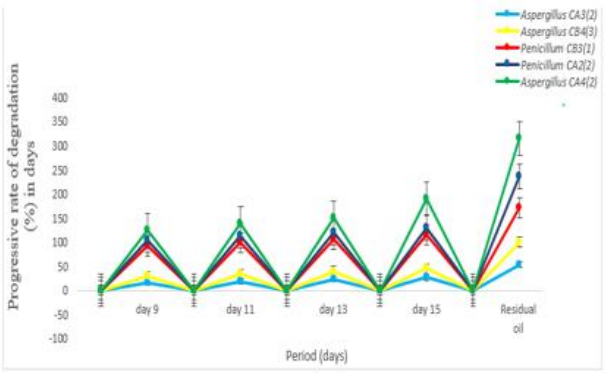

Figure 1b: Progressive rate of degradation (%) of spent engine oil after 15 days

Table 4: Progressive decrease in the weight of spent oil for 7 days

Isolate Code	Weight (g)				Residue Oil (g)
	DAY 1	DAY 3	DAY 5	DAY 7	
CA3(2)1	299.11	299.00	298.71	298.42	2.01
CB4(3)1	273.59	273.38	273.27	273.24	3.05
CB3(1)1	265.47	265.38	265.33	265.19	2.11
CB3(3)1	254.97	254.87	254.69	254.41	2.08
CA2(2)1	305.04	304.93	304.81	304.61	1.92
CA4(2)1	273.11	273.02	272.71	272.51	1.99
MEDIA CONTROL B1	222.30	222.30	222.30	222.30	0.00
MEDIA CONTROL B2	234.44	234.44	234.44	234.44	0.00
OIL CONTROL B1	199.41	199.41	199.41	199.41	3.17
OIL CONTROL B2	240.67	240.67	240.67	240.67	3.17

The decrease in weight of oil inoculated continued decreasing progressively from 3.55g to 1.3g, 1.17 and 1.09g at day 0, day 9, day 13 and day 15 respectively (Figure 1b). The result revealed that on day 9, 11, 13, 15 for isolate CA3 2(2) there was (15%, 18.3%, 22% and 26%) degradation respectively, change in amount of the spent engine oil and rate of degradation were significant at $p \geq 0.05$, while the controls remained constant (Table 5).

Table 5: Progressive decrease in the weight of spent oil for 15 days

Isolate Code	Weight (g)				Residue Oil (g)
	DAY 9	DAY 11	DAY 13	DAY 15	
CA3(2)2	243.13	243.04	242.95	242.81	1.62
CB4(3)2	245.57	245.51	245.43	245.36	2.21
CB3(1)2	250.81	250.73	250.62	250.51	0.99
CB3(3)2	282.50	282.42	282.37	282.29	2.61
CA2(2)2	277.56	277.54	277.51	277.46	1.22
CA4(2)2	288.31	288.27	288.14	288.04	0.63
MEDIA CONTROL B1	222.30	222.30	222.30	222.30	0.00
MEDIA CONTROL B2	234.44	234.44	234.44	234.44	0.00
OIL CONTROL B1	199.41	199.41	199.41	199.41	3.55
OIL CONTROL B2	240.67	24.67	240.67	240.7	3.55

The residual spent engine oil result revealed that the rate of degradation as compared between day 7(1st extraction) and day 15 were significantly different (Figure 2). There was loss of many peaks and bands from the GCMS result when compared with the undegraded oil (Figure 3).

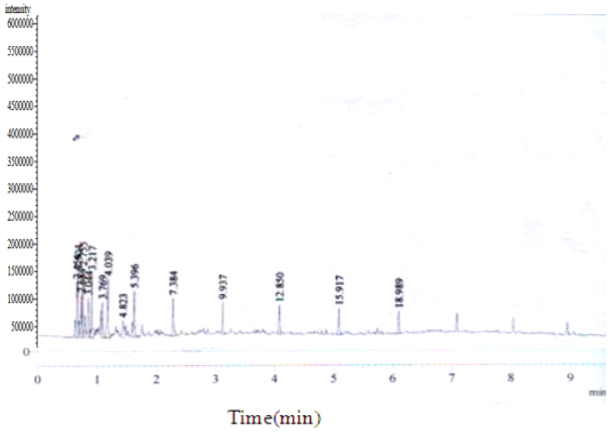

Figure 3a: Spent engine oil before degradation

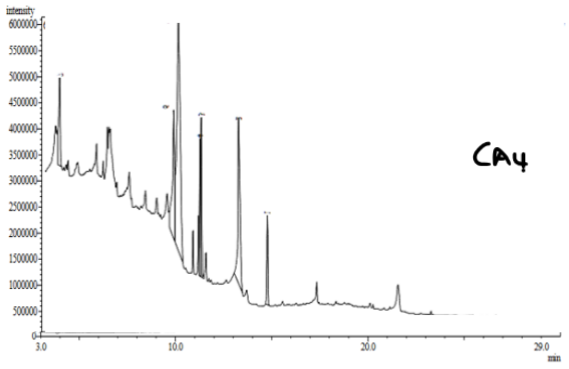

Figure 3b: Spent engine oil after 15days exposure to Aspergillus sp CA4(2)

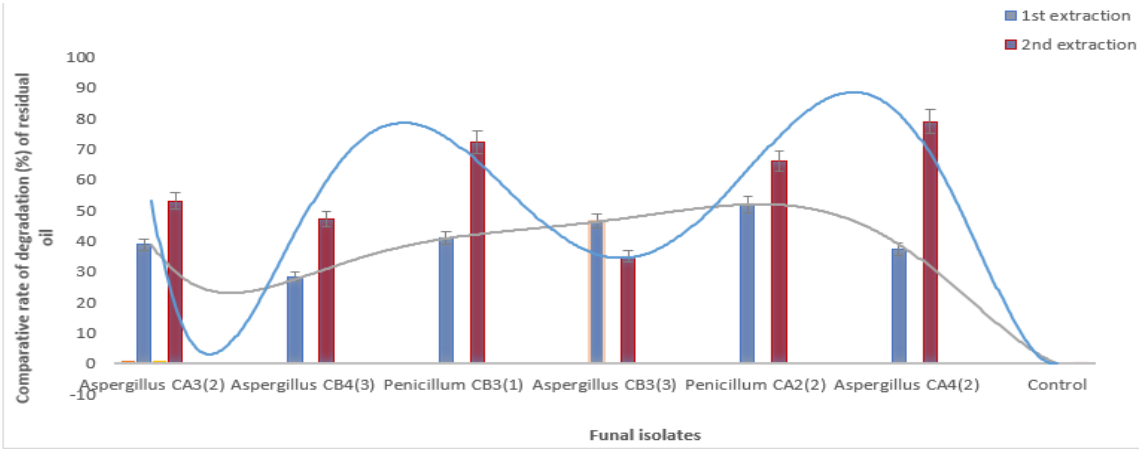

Figure 2: Comparative analysis between day 7 residual oil and day 15 residual oil

DISCUSSION

In sample CA, the highest recorded CFU (7.4×10^{-4} CFU/g) shows that there is a high microbial population capable of degrading hydrocarbons, likely due to the presence of microorganism that have adapted to the used motor oil contaminated site. The lowest CFU (2.2×10^{-9} CFU/g) show a less favorable environment and microbial population. This may be due to the fact that sample CA5 and CB5 were the last dilution factor after serial dilution.

In this study, thirty pure fungi isolate with different morphological characteristics was isolated from the two-sampling site. Table 3 listed all the fungal isolates that were identified and characterized morphologically. The fungal isolates showed difference in morphological appearance, pigmentation and sporulation in the media used. Based on the macroscopic and microscopic morphological characteristics, six of the fungal isolates belong to the genera *Aspergillus spp.* and *Penicillium spp.* respectively. Among all the fungal isolates obtained Aspergillus species were the most common, these aligns with the findings of Thenmozhi *et al.,* (2013) who reported that filamentous fungi can grow on hydrocarbons with *Aspergillus*

sp. being the most frequent (Thenmozhi *et al.,* 2013). In addition, Okerentugba and Ezeronye (2003) demonstrated that *Penicillium spp., Aspergillus spp.* and *Rhizopus spp.* were capable of degrading hydrocarbons especially when single cultures were used. Batelle (2000) showed that fungi Aspergillus were better degraders of hydrocarbon.

Biodegradation study took place for a period of 15 days and was monitor at interval of 2 Days starting from Day 1. From the study it was observed that *Penicillium* CA2(2) has the highest degradation rate of 51.9% after the first extraction at Day 7, followed by *Aspergillus* CB3(3) (46.5%), *Penicillium* CB3(1) (41.5%), and the least recorded was *Aspergillus sp* CB4(3) which recorded (28.2%). It was observed after the first seven days of degradation the residual oil from mixture exposed to *Penicillium* CA2(2) recorded the highest degradation rate (51.9%) and the least was *Aspergillus* CB4(3) (28.2%) (Figure 2); At the Day 15 of extraction, which was the second phase of the extraction it was observed that the isolate of *Aspergillus* CA4(2) has the highest degradation rate of 78.9%, followed by *Penicillium* CB3(1) which recorded 72.1% and the least was *Aspergillus* CB3(3) at 34.9% (Figure 2). Similar result was reported by the study of Behera *et al.* (2019) who recorded that that *Penicillium chrysogenum* effectively degraded various hydrocarbons at a rate of 57-70%, this finding is in line with the rate of degradation achieved by *Penicillium* CB3(1) used in this study. It supports the finding of Kumar *et al.,* (2023), where *Penicillium* isolates were reported to achieve notable degradation rates.

The lower degradation rate of *Aspergillus spp.*CB3 (3) at 34.9% suggests that not all *Aspergillus* strain shows the same level of efficiency in biodegradation. Factors such as difference in genetic, metabolic pathways, and environmental conditions can influence the degradation capabilities of these fungi (Deshmukh *et al.,* 2016; Elemba *et al*., 2019). Certain strains may lack the necessary enzymes or metabolic adaptations to effectively degrade specific substrates. The result of the biodegradation revealed that of *Aspergillus* CA4(2) fungal strain was more efficient with 78.9% degradation and hence the residual spent engine oil resulting from biodegradation of *Aspergillus* CA4(2) was measured using the gas chromatography and mass spectroscopy technique (GC-MS), (Figure 3). The result (Figure 3a) revealed the loss of many peaks, complete removal of pentadecane and 2,6,10-trimethylpentadecane when compared with untreated spent engine oil (Figure 3b).This result was similar to the findings of Udeme *et al.* (2023) which showed that *Aspergillus versicolor* and *Aspergillus niger* exhibited biodegradation of hydrocarbons with reduction in 4 peaks and emergence of new peak not present in the undegraded oil.

CONCLUSION

The results obtained in this study has shown that axenic culture of *Aspergillus spp.,* and *Penicillium spp.,* from used engine oil contaminated soil samples can be exploited in the biodegradation of petroleum oil spill and bioremediation of the environment.

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