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Physicochemical and Fungal Analysis of a Hydrocarbon-Polluted Soil at Amadi-Ama Creek of Bonny River Port Harcourt, Rivers State, Nigeria

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Abstract

Numerous hydrocarbon-utilizing fungal species have been implicated with the ability to utilize/degrade hydrocarbon as carbon source, which indicate their potential for environmental cleanup in hydrocarbon-contaminated sites. In this study, five (5) indigenous fungal species were isolated from a petroleum-hydrocarbon polluted soil at Amadi-ama Creek, Bonny river shoreline, Port Harcourt, Rivers State. These fungal species may have high potential to biodegrade petroleum hydrocarbon pollutants. Samples were collected randomly from the hydrocarbon impacted soil at Amadi-ama Creek, Bonny river shoreline. Sabouraud's Dextrose Agar (SDA) and Czapek Agar were used

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as growth media. Samples were examined to assess the physical and chemical characteristics such as conductivity, pH, temperature, nitrate, iron, copper, zinc, chromium, phosphate, sulphate, total hydrocarbon content, total petroleum hydrocarbon, and polycyclic aromatic hydrocarbon. These parameters are known to influence the occurrence, diversity and distribution of microorganisms in an ecological niche. Soil pH showed to be acidic (6.21), result also showed that Total hydrocarbon content, Total petroleum hydrocarbon and Polycyclic aromatic hydrocarbon values were at 306.55mg/kg, 112.134mg/kg, 44.227mg/kg respectively establishing the incidence of hydrocarbon pollution. Electrical conductivity and Temperature were at 1250 Us/cm, and 29.58^{0}C respectively while Nitrate, Phosphate, Sulphate, Iron, Copper, Zinc, and Chromium values were at 1.228mg/kg, 0.751mg/kg, 20.214mg/kg, 18.431mg/kg, 0.113mg/kg, 0.121mg/kg, and 0.042mg/kg respectively. Monitoring of the soil quality established the incidence of hydrocarbon pollution as well the incidence of anthropogenic influence on the soil putting into cognizance human activities at the shoreline. Morphological identification of obtained fungal species led to their assignment into four (4) genera and five (5) species; *Aspergillus niger* (28%), *Aspergillus flavus* (22%), *Cladosporium herbarum* (20%), *Penicillium notatum* (17%), *Fusarium spp* (11%). The predominance of *Aspergillus* isolates (28% and 22%) in this study could be a pointer to their potential to utilize hydrocarbon as their sole source of nutrient. The isolation of these indigenous fungal species could serve as a baseline study on which further analysis such as hydrocarbon degradation screening test could determine their individual hydrocarbon degradation potentials and subsequent consideration as hydrocarbon degrading microbes in subsequent bioremediation study.

Keywords: Indigenous fungi, Bonny Estuarine, Soil, Petroleum-Hydrocarbon, Bioremediation

INTRODUCTION

Hydrocarbon are compounds composed entirely of carbon and hydrogen. Aromatic hydrocarbon, alkanes, alkenes, cycloalkanes, alkynes, and combinations of these compounds comprise different types of hydrocarbon. Complex mixtures of hydrocarbon occur naturally in crude oil and gasoline. Most can be used as substrates in metabolism by bacteria, archaea, fungi, and algae (Abbasian *et al.,* 2015, Rabus *et al.,* 2016). While fungi and algae degrade hydrocarbon aerobically, bacteria and archaea are capable of both aerobic and anaerobic degradation (Xue *et al.,* 2015). A naturally occurring mixture of hydrocarbon and non-hydrocarbon molecules, including paraffinic, naphthenic, and aromatic compounds, found deep beneath the Earth's surface is called petroleum, often referred to

as crude oil. Soils contaminated by petroleum is a critical and challenging environmental issue in various locations, especially in soil where contamination tends to accumulate significantly (Cabrerizo *et al.,* 2011). This contamination includes a diverse group of lipophilic organic compounds known as polycyclic aromatic hydrocarbons (PAHs), composed of two or more fused aromatic rings (Abdel-Shafy *et al*., 2016).

Microorganisms play diverse roles in biotechnology; one of such roles is bioremediation (the use of living things in the cleanup of polluted environments) (Silas *et al.,* 2023; Martins *et al.,* 2024). Bacteria and Fungi are among the major groups of microorganisms widely used in biotechnological applications, the former are easily manipulated genetically while the latter exhibit diverse growth pattern such as secretion of extracellular enzymes and invasive mode of growth (Timothy *et al.,* 2022). Artificial and natural release of petroleum and related products into the environments endanger aquatic and terrestrial life forms (Olawale *et al.,* 2021). Implications of such release include: devegetation, contamination of potable water sources, fall in reproduction of both plants and animals due to disruption in food chain, and death of plants and animal inhabiting the polluted environments (Chikere and Azubuike, 2013; Abah *et al.,* 2021; Emmanuel *et al.,* 2021). The bioremediation of hydrocarbon impacted soil can be promoted by stimulating the native microbial community through the addition of nutrient or oxygen (biostimulation) or through inoculation with an appropriate microbial consortium(bioaugmentation), (Seklemova *et al.,* 2015).

According to existing literature, various approaches have been employed to achieve bioremediation of total petroleum hydrocarbon mixtures using various microorganisms including bacteria, fungi, algae, and plants (Tang *et al.,* 2012). However, fungi are the most potential microorganisms in terms of biodegradation of petroleum hydrocarbon and other recalcitrant compounds, these abilities are due to their unique characteristics such as diversity (ubiquitous), morphology, versatility, and metabolic capabilities, hence fungi became suitable for bioremediation of hydrocarbon pollutants including the PAHs (Chikere and Azubuike, 2013). Furthermore, a previous study on hydrocarbon contaminated soil showed that fungi are more effective than bacteria in the hydrocarbon degradation, because the reported biodegradation efficiency ranged from 6-82% for fungi and 0.13-50% for bacteria (Jeong *et al.,* 2015). The ability to degrade high molecular weight hydrocarbon pollutants depends on some advantages that were found in fungi. These include; secretion of low substrate-specific enzymes, ability to grow in an extreme

environment than bacteria, and more access to hydrocarbon contaminants due to the formation of the mycelial network (Abbasian *et al.,* 2015). Fungi has been reported to produced biosurfactants which serve as a mechanism for achieving biodegradation of hydrocarbon, however, this is not open to all but rather some specific fungal organisms (Rabus *et al.,* 2016). Previous studies indicated that indigenous fungi belonging to the genera *Cladosporium*, *Alternaria*, *Fusarium*, *Trichoderma*, *Aspergillus*, *Penicillium*, *Paecilomyces*, *Coriolus*, *Pycnoporus*, *Pleurotus*, *Cephalosporium*, *Mucor*, *Fomitopsis*, and *Daedalea* are responsible for degrading of PAHs and diesel oil in the soil and aquatic environments (Marchand *et al.*, 2017).

Currently, scientists have made significant attempts to identify fungi adapted to oil-contaminated environments that can degrade PAHs, the recalcitrant fractions of petroleum and crude oil, since they are critical components in impacting the success rate of bioremediation (Benguenab and Chibani, 2021). Presently, most native fungal isolates were investigated to determine their ability to degrade individual PAHs, which existed in crude oil, but comprehensive information on the biodegradation level of total petroleum hydrocarbons (TPHs) is scarce (Fallahi *et al.*, 2023). This study had the objective of isolating and characterizing fungal species from a hydrocarbon impacted soil at Bonny estuarine in Amadi-ama Creek Port Harcourt River state, enumeration of the isolated fungal species as well review of their individual percentage of occurrence in the sampled site. Subsequently, morphological assessments to classify isolates into genera were conducted.

MATERIALS AND METHODS

Study Area

The study area considered for this research is Amadi-Ama Creek which is located in Port Harcourt Local Government Area of Rivers State and lies between longitude 07° 02' 57.627"E and latitude 04° 48' 51.113"N. The creek is one of the tributaries of the upper Bonny Estuary, brackish and tidal in nature with fresh waters intrusion from the surrounding inland waters and flood during the wet season. The Bonny River Estuary lies on the South-Eastern edge of the Niger Delta between longitudes $6^0$58'and $7^0$14'East and latitudes $4^0$19' and $4^0$34'North with an estimated area of 206km2 and extends 7km offshore to a depth of about 7.5metres (Scott, 1966).

Sample Collection

Hydrocarbon polluted soil samples were used for the analyses. Soil samples from two random points were aseptically collected with sterile containers from hydrocarbon-impacted area of Amadi-ama Creek, Port Harcourt, Rivers State and labelled accordingly. The samples collected were transported aseptically in steriled containers and ice chest to BGI Laboratories Ltd in Port Harcourt, Rivers State and Microbiology laboratory, Faculty of Pure and Applied Sciences, Federal University Wukari, Wukari city, Taraba State for physicochemical analysis and microbial analysis respectively.

Determination of Physicochemical Properties

The pH, temperature and electrical conductivity of the soil samples were determined using electrometric method, employing the use of HI 9289 digital multiparameter meter manufactured by Hanna Instruments. The total hydrocarbon content of the soil was determined using infrared spectroscopy, following soxhlet extraction with tetrachloroethylene (TCE) solvent. For total petroleum hydrocarbon determination, n-hexane was used in the soxhlet extraction and anhydrous sodium sulphate was deployed in the fractionation. The eluent was then injected into the inlet of gas chromatograph (GC) and detected using flame ionization (FID). For the determination of polycyclic aromatic hydrocarbon, the soxhlet extraction was carried out using dichloromethane (DCM) solvent and injected into the gas chromatograph (GC) for detection by mass spectrometry (MSD). Determination of nitrate, phosphate and sulphate was carried out using UV/VIS spectrophotometry, wherein their various standards were used for calibration, and absorbance determined at their respective wavelengths. For the determination of metals; iron, copper, zinc and chromium, their respective standards were used for the calibration on the atomic absorption spectrophotometer (AAS), wherein the holocathode lamps for each element was also deployed (Al-hawash *et al.,* 2018).

Fungal Isolation

Soil samples contaminated with crude oil from Amadi-ama Creek Port Harcourt in River state, Nigeria were collected with an auger, labelled and sent to Microbiology Department, Federal University Wukari, Taraba state in Nigeria to identify specific fungi indigenous to

the crude oil exposed shoreline. Sampling was conducted on a sunny day with a temperature of 32 °C. Samples weighing 500 g were taken from three random points within the contaminated shoreline. Sampling was performed randomly at three depths; 0 cm, 10 cm, and 20 cm. The collected subsamples were thoroughly mixed to create one sample from each dept. These samples were sieved through a 2.5 mm mesh to remove stones, and the sieved soils were air-dried at room temperature for use in the fungal isolation procedure. Fungal isolation was carried out using a dilution plate technique. One gram of soil sample was added to a 0.05% Water Agar (WA) medium, prepared by mixing 0.5 g of agar in 1 L of water and autoclaving it at 121 °C for 15 min. Subsequently, 1 ml of a 10-times diluted soil suspension was spread onto Sabouraud Dextrose Agar (SDA), and Czapek Agar media. These plates were then incubated at 25°C for 7 days, and the developed colonies on each plate were transferred to fresh SDA plates to obtain axenic cultures (Timothy *et al.,* 2022).

Fungal identification

Fungal identification involved a combination of morphological characterization, encompassing features such as conidiomata, conidiophores, and conidiogenous cells. various taxonomic keys were employed to morphologically distinguish each fungal isolate (Timothy *et al.,* 2022).

RESULTS

Determination of Physicochemical Properties of Soil Samples and Identification of Fungal Species

In this Study, culture-dependent screening of hydrocarbon-polluted soil samples revealed different genera of fungi which might have utilized hydrocarbon as a carbon source. The physical and chemical characteristics of hydrocarbon polluted soil samples collected is presented in table 2 below. The soil samples were also analysed for indigenous fungi by plating on SDA and Czapek medium. A Total of five (5) fungal isolates were obtained, and they covered four fungal genera namely: *Aspergillus, Penicillium, Cladosporium, and Fusarium* (Tables 1). It was observed that *Aspergillus niger* and *Aspergillus flavus* dominated with 28% and 22% frequency of occurrence respectively (Figure 1). The fungal counts obtained from the soil sample (cfu/g) ranged from $8.0x10^4$ to $3.0x10^6$cfu/g and $1.0x10^4$ to $2.0x10^6$cfu/g for SDA and Czapek medium respectively (Tables 3). The soil sampled site were found to

be richer with *Aspergillus* spp. than any other fungal genera whereas *Fusarium* spp. was non dominant (Tables 4). Similarly, two species of *Aspergillus* were isolated from the two soil sampled points thus their high prevalence (Table 4).

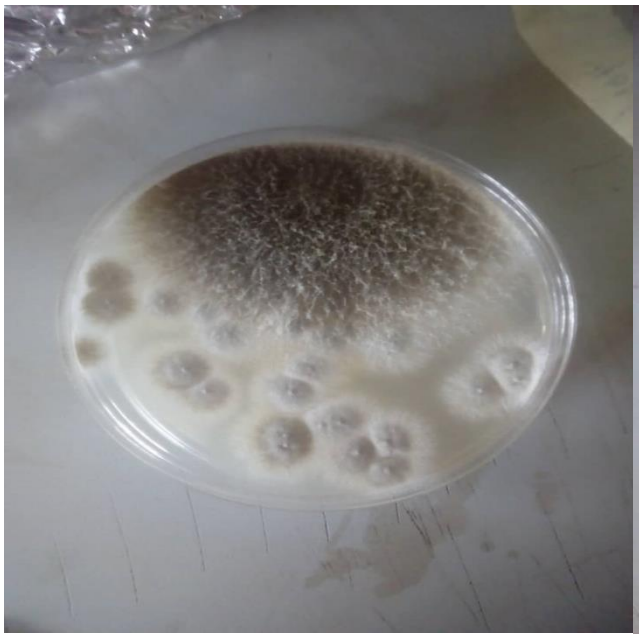

Plate 1. Isolate of *Aspergillus niger (SDA)*

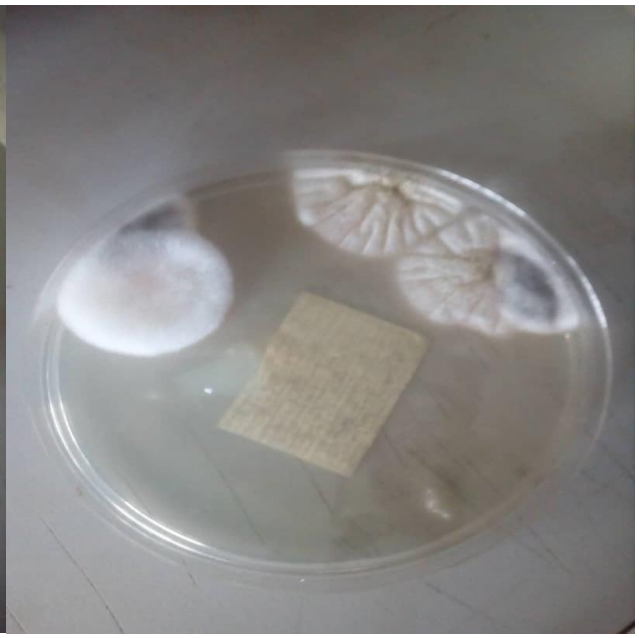

Plate 2. Isolate of *Penicillium notatum (SDA)*

Table 1. Cultural and cell morphology of fungal isolates obtained from hydrocarbon-polluted soil samples

Tentative isolates	Size	Microscopic Characteristics	Margin	Elevation	Colonial Appearance
Aspergillus Niger	Large	Simple septate and branched conidia in chains.	Entire	Flat	Black Powdery with radial fissures
Penicillum Notatum	Medium	Septate hyphae with branched conidiophores attached to metula. Flask-chains or round conidia are attached to the metula.	Entire	Raised	Blue-green with a yellowish pigment Fluty with radial fissures
Cladosporium herbarum	Large	Hyphomycete forming branched acropetal chains of conidia, each with a distinct hilium.	Entire	Raised	Blackish-brown Powdery and smooth conidiophore

Aspergillus flavus	Large/small	Short conidiophores with rough walls. Phialides are both Uniseriate and biseriate, cover the entire vesicle, and point out in all directions.	Entire	Raised	Yellowish-green Conidial with radial fissures
Fusarium spp	Large	Small, oval microconidia mixed with smaller numbers of crescent shaped macroconidia.	Entire	Flat	Whitish White smooth and shinning

Table 2. Physicochemical properties of sediments

S/N	Parameters	Unit	Results
1.	pH	-	6.21
2.	Total hydrocarbon content (THC)	mg/kg	306.55
3.	Total petroleum hydrocarbon (TPH)	mg/kg	112.134
4.	Polycyclic aromatic hydrocarbon (PAH)	mg/kg	44.247
5	Electrical conductivity	µS/cm	1250
6.	Temperature	°C	29.58
7.	Nitrate	mg/kg	1.228
8.	Phosphate	mg/kg	0.751
9.	Sulphate	mg/kg	20.214
10.	Iron	mg/kg	18.431
11.	Copper	mg/kg	0.113
12.	Zinc	mg/kg	0.121
13.	Chromium	mg/kg	0.042

Table 3. Fungal counts from hydrocarbon-polluted oil samples

Sample	Dilution factor	SDA	CZAPEK
SSP1	10^4	8.0×10^4	1.0×10^4
SSP2	10^6	3.0×10^6	2.0×10^6

Table 4. Frequency of isolated fungi species with corresponding percentage occurrences

S/N	Tentative Isolates	Frequency	Percentage occurrence
1	*Fusarium spp*	4	11%
2	*Penicillium notatum*	6	17%
3	*Cladosporium herbarum*	7	20%
4	*Aspergillus flavus*	8	22%
5	*Aspergillus niger*	10	28%

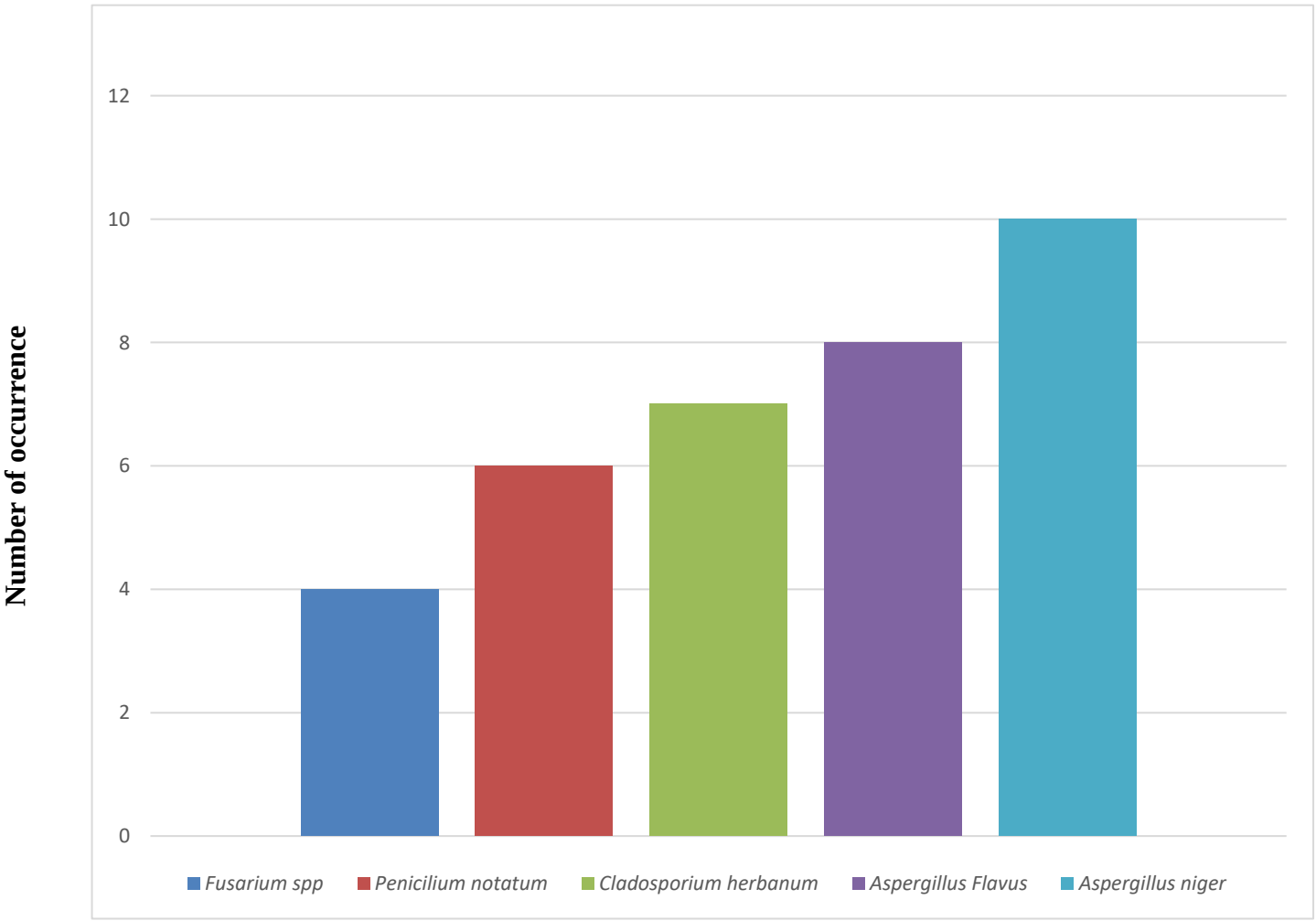

Figure 1. Percentage occurrence of fungal isolates obtained from hydrocarbon-polluted soil samples

DISCUSSION

Complex mixtures of hydrocarbon occur naturally in crude oil and gasoline. Most can be used as substrates in metabolism by bacteria, archaea, fungi, and algae (Abbasian *et al.,* 2015; Rabus *et al.,* 2016). While fungi and algae degrade hydrocarbon aerobically, bacteria and archaea are capable of both aerobic and anaerobic degradation (Xue *et al.,* 2015).

The fungal genera isolated from hydrocarbon-polluted soil samples in this study have been implicated in degradation of hydrocarbon such as crude oil, polyaromatic hydrocarbon and refined petroleum (Chikere and Azubike 2014). The growth of the fungal species in the laboratory could be attributed to their ability to utilize hydrocarbon or any other alternative nutrient in the sample as carbon source. The predominance of *Aspergillus niger* could point to the fact that the organisms possess an enzyme system that makes the organism thrive in an intense hydrocarbon impacted shoreline as revealed from the physical and chemical characteristics of the sampled site. This result is in tandem with the findings of Igiebor et al. (2017).

The physical and chemical properties of the hydrocarbon-polluted soil samples established that the study site is heavily polluted with hydrocarbons. It also suggests that under optimal conditions, the isolated fungal species with emphasis on *Aspergillus niger* (Plate 1) could possess the ability to degrade hydrocarbons in soils, and can be considered for hydrocarbon degradation screening test in a bioremediation study (Table 4, Figure 1). Monitoring of the soil quality established the incidence of hydrocarbon pollution, as well as the incidence of anthropogenic influence along the shoreline, and on the soil (Table 2). These parameters are known to influence the occurrence, diversity, distribution and abundance of microorganisms in an ecological niche.

The enumeration of these indigenous fungal species for soil sample point 1 (SSP_1) and soil sample point 2 (SSP_2) by viable counts technique from the soil sample (cfu/g) ranged from $8.0x10^4$ to $3.0x10^6$cfu/g and $1.0x10^4$ to $2.0x10^6$cfu/g for SDA and Czapek medium respectively revealing the abundance of these species in such petroleum-hydrocarbon exposed environment thus can be screened for hydrocarbon degradation potentials making them potential species for future hydrocarbon bioremediation studies (Tables 3).

The presence of fungal species in the contaminated soil demonstrates their adaptability to the harsh conditions of hydrocarbon pollution. This adaptability makes them valuable candidates for bioremediation processes. The ability of these fungi species to utilize

hydrocarbon as carbon source indicate their potential for environmental cleanup in hydrocarbon-contaminated sites (Asemoloye *et al.,* 2015). While more research is needed to fully understand the mechanisms by which these fungi species employ in breaking down hydrocarbons in soils, the findings from this study have shown that fungi may be a valuable tool for bioremediation of contaminated sites. Furthermore, the study highlights the importance of preserving and protecting fungal diversity, as these organisms may be critical for maintaining healthy system. This suggests that these fungi may play a role in the natural attenuation of petroleum pollutants. For example, the study found a number of isolates belonging to the genera *Aspergillus spp, Penicillium notatum* (Plate 2)*,* and *Aspergillus Flavus* which are known to be versatile degraders of hydrocarbon, and they have the ability to produce enzymes that can break down a wide range of pollutants.

CONCLUSION

This study demonstrated that, hydrocarbon degrading organism could be isolated from hydrocarbon polluted area and *Aspergillus niger* was found to be predominant among the fungi isolated. This could be suggested that, *Aspergillus niger* could have a potential to be used as hydrocarbon degrading organism upon a successful screening test for hydrocarbon degradation, thus can be considered in bioremediation for hydrocarbon contaminated areas. The findings from this research suggest that fungi may play an important role in the bioremediation of hydrocarbon-contaminated environments if properly studied in view of their hydrocarbon degradation potentials. The diversity of fungal species that have been identified in this study, as well as other studies, highlight the potential of fungi to degrade a wide range of hydrocarbon. Given this potential, it is recommended that future research focus on molecular isolation and characterization of fungal species from hydrocarbon-contaminated environments as well hydrocarbon screening assay, with the goal of identifying fungal strain that can be used in bioremediation efforts or programs.

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Competing Interest

Authors have declared that no competing interests exist.

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