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Diversity of Fungi Isolated from Iraqi Petroleum-Contaminated Soils (Baiji Refinery and Middle Oil Company)

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Abstract

Fungi are important decomposers of complex organic pollutants and have an enormous potential for use in bioremediation of petroleum-laden environments. In the current study, we investigated the diversity, distribution, and oil-degrading potential of fungi isolated from petroleum-contaminated soils in Iraq, specifically Baiji Refinery and Middle Oil Company. Fifteen soil samples were collected from each location as outlined between the months of September and November 2024. Fungal isolates were purified and identified based on morphological and microscopic examinations of the fungi using standard taxonomic keys. A total of 232 isolates were obtained, largely belonging to Ascomycota, with the predominant species being *Aspergillus flavus*, followed by *Cladosporium* sp., *Penicillium griseofulvum*, and *Candida* spp. quantitatively analyzed patterns revealed a significantly different frequency and occurrence of fungal isolates among each of the locations, which suggest that these species adapted to their hydrocarbon-rich environments. The presence of these oil-tolerant species suggests their potential enzymatic capabilities for the degradation of petroleum hydrocarbons and the ecological role they play in contaminated ecosystems. This is the first systematic study with culturable oil-degrading fungi from Iraqi petroleum-contaminated soils for basic research and applied bioremediation efforts using native fungal strains.

Keywords: Fungal isolation, petroleum-contaminated soil, hydrocarbon degradation.

1. Introduction

Soil is a dynamic ecosystem including different microorganisms that have vital roles in nutrient cycling and degradation[1]. However, petroleum soil has more contamination that could disrupt microbial communities and pollute the environment. The types of microorganisms that live in agricultural soils are different from those that live in oil-soaked soils, and the reason for this is the different physical and chemical properties of these soils[2,3].

Oil-contaminated soils are soils that contain oil products when oil is spilled on them from tanks or during extraction, refining, or transportation processes[4,5]. Agricultural soils are natural soils that contain minerals in a balanced ratio suitable for plant life, and they differ from soils contaminated with oil products in terms of permeability, water retention capacity, and aeration[6]. Environments contaminated with hydrocarbons (oil and its derivatives) host a wide variety of microorganisms, such as fungi, yeasts, and even bacteria that adapt and metabolize to survive in this environment, using hydrocarbons as an energy source. Current studies mostly focus on temperate regions and/or single fungal species, leaving gaps in understanding the diversity and bioremediation potential of local fungi in oil-rich ecosystems[7, 8].

Though the petroleum industry in Iraq has a long history and is known for frequent oil spills, little research has examined the indigenous fungal populations in oil-contaminated soils or their ability to bioremediate the contaminated soils. The physicochemical conditions of Iraqi petroleum soils (high soil salinity, high temperature variabilities, and petrochemical loads) create an exceptional ecological niche for novel or highly adapted fungal taxa. Consequently, studying these ecosystems has potential scientific value for understanding fungal diversity and biodegradation potential under extreme local conditions.

Fungi are important organisms with a high ability to produce various enzymes, acids, and other compounds of significance in several areas[9], such as industrial and agricultural aspects and the production of medicines[10]. Since crude oil contains high levels of aromatic, aliphatic, resinous, and asphaltic products, these materials are hazardous to humans and animals[11,12]. Fungi exploit their ability to decompose as they are used in the degradation of hydrocarbons, and therefore they can be used to clean the environment from oil waste[13]. Treating the environment with fungi is considered one of the safest methods, as these pollutants are not extracted but are instead decomposed naturally by these organisms[14]. Many fungal species have been isolated from oil-contaminated soils, which have been proven through the studies to have the ability to degrade oil pollutants such as *Cephalosporium*, *Aspergillus*, *Alternaria*, *Monilia*, *Geotrichum*, *Cladosporium*, *Rhizopus*, *Penicillium*, *Mucor*, *Candida*, *Thamnidium*, *Torulopsis*, and *Rhodotorula*[15]. It has also been demonstrated that the yeast *Saccharomyces* can degrade polycyclic aromatic hydrocarbons into simpler, non-toxic forms[16].

The aim of this study was to isolate and identify fungal species from petroleum-contaminated soils from the Baiji Refinery and the Middle Oil Company and assess their hydrocarbon-degrading potential for future bioremediation techniques.

2. Materials and Methods

2.1. Collecting samples

From oil soil areas located in Iraq (Baiji Refinery and Middle Oil Company), samples were collected during the period from September to November 2024; the sampling sites were georeferenced using GPS, and environmental parameters such as pH, temperature, and total petroleum hydrocarbons (TPH) concentration were recorded to examine. Fifteen samples were collected from each site, placed in sterilized bags, and transported to the Advanced Mycology Laboratory of the Department of Biology at the College of Education for Pure Science/ Ibn Al-Haitham[17]. As the most published literature lacks comprehensive data on fungi in Iraqi oil-contaminated areas. Moreover, few studies link morphological identification of isolates to their practical applications in bioremediation. This study aims to isolate and identify types of fungal species from petroleum-contaminated soil and evaluate the potential fungi for hydrocarbon degradation for future bioremediation techniques.

2.2. Isolation and identification of fungi

A dilution method was used where an equivalent of 50 g of soil was weighed and then placed in a container containing 250 ml of sterile distilled water. It was then placed on a mixer for 5 min. Thereafter, 1 ml of the suspension was taken and added to 9 ml of distilled water to achieve a dilution of 10^2. Subsequently, 1 ml of the second suspension was taken and added to 9 ml of distilled water to achieve a dilution of 10^3 according to [12]. One ml of the solution was placed on potato dextrose agar (PDA) plates supplemented with 20 µg of tetracycline to prevent bacterial growth and incubated at a temperature of 25±2ºC for seven days. Then, it was purified by taking a spore from the edge of each colony and incubated for seven days. The samples were identified microscopically using lactophenol blue staining and classified using available taxonomic keys. All isolation procedures were performed in triplicate for reproducibility and also contained sterile controls for monitoring contamination. Identification of fungi was carried out using standard taxonomic keys described by [18-20] based on macroscopic and microscopic characteristics. The pure isolates were preserved in the Mycology Laboratory of the Department of Biology, College of Pure Sciences Ibn Al-Haitham, University of Baghdad[21].

2.3. Measuring the appearance and frequency percentages

Measuring the appearance and frequency percentages for each type, where the frequency and appearance of each type were measured using the following **Equations**[22]:

$$Percentage\ of\ oocurance \frac{Number\ of\ isolates\ of\ a\ species}{Total\ number\ of\ isolates} \times 100 \quad (1)$$

$$Percentage\ of\ frequency \frac{Number\ of\ samples\ containing\ a\ specie}{Total\ sample} \times 100 \quad (2)$$

3. Results

A grand total of 232 fungal isolates from the contaminated soils were grouped into 12 genera. Statistical tests analyze species frequency and distribution and indicate site-specific adaptation. While no molecular identification or diversity indices were made, the distribution patterns suggest notable ecological differentiation across sites. The results showed that 232 isolates were obtained from soils contaminated with petroleum products. The majority of the isolates belong to the phylum Ascomycota, while the least belong to the phylum Mucoromycota. As shown in **Table 1**.

Table 1. Shows the number, species, and source of the isolated fungi.

The name of the isolated fungi	**Middle Oil Company (15 isolates)**	**Beiji Refinery (15 isolates)**	**Private generators (10 isolates)**	**Total**	**Frequency%**	**Appearance%**
Cladosporium sp.	10	13	0	23	9.91	57.5
Curvularia sp.	4	7	2	13	5.6	32.5
Aspergillus flavus	10	8	9	27	11.6	67.5
Aspergillus ochraceus	10	6	6	22	9.4	55
Alternaria alternate	6	4	8	18	7.7	45
Asperillus niger	6	6	5	17	7.3	42.5
Pencillum grisofulvum	6	10	7	23	9.9	57.5
Aspergillus nidulans	5	0	8	13	5.6	32.5
Fusarium solani	4	7	5	16	6.8	40
Aspergillus caespitous	0	0	2	2	0.8	5
color Yeast (yellow, orang, pink)	5	4	7	16	6.8	17
Candida spp.	9	9	5	23	9.9	57.5
White sterile hyphae	9	8	2	19	8.1	47.5
Total	84	82	66	232		

The fungus *Aspergillus flavus* (**Figure 1**) was the most frequent and prominent among all isolates, with a frequency of 11.6 and a presence of 67.5. *Cladosporium* sp. (**Figure 2**) it was present at a frequency of 9.91% with an appearance of 57% in oil-contaminated soil, *Aspergillus ochraceus* (**Figure 3**) had a frequency of 9.4% and an occurrence of 55% for oil-contaminated soil, while for agricultural soil, it had a frequency of 8.6% and an occurrence of 80%, *Pencillum grisofulvum* (**Figure 4**) appeared with a frequency of 9.9% and an incidence of 57.5% in oil-contaminated soil, *Fusarium solani* (**Figure 5**) had frequencies of 6.8% and 40% respectively, *Alternaria alternate* (**Figure 6**) had a frequency of 7.7% and an appearance rate of 45%

concerning the oil soil. *Aspergillus niger* (**Figure 7**) had a frequency of 7.3% and an appearance of 42.5%., the white yeast *Candida* spp. (**Figure 8**) had a frequency of 9.9% and an appearance of 57.5% in oil-contaminated soil, while colored yeast had a frequency of 6.8% and 17% respectively for the oil soil, while the agricultural soil had a frequency of 6.5% and an appearance of 60%, as shown in **Figure (8)**, *Curvularia* sp. (**Figure 9**) had a frequency of 5.6% and an occurrence of 32.5% in oil-contaminated soil, while in agricultural soil, it had a frequency of 8.6% and an appearance of 80%, *Aspergillus nidulans* had a frequency of 5.6% and an appearance rate of 32.5% in oil-contaminated soil, as shown in **Figure (10)**, and *Aspergillus caespitous* had a frequency of 0.8% and an appearance of 5% in oil soils **(Figure 11)**. *Rhizopus stolonifer* also did not appear in oil soil, while it was found in agricultural soil with a frequency and appearance of 4.3% and 40%, respectively, and *Fusarium oxysporum* has a moderate growth rate as shown in **Table 1**.

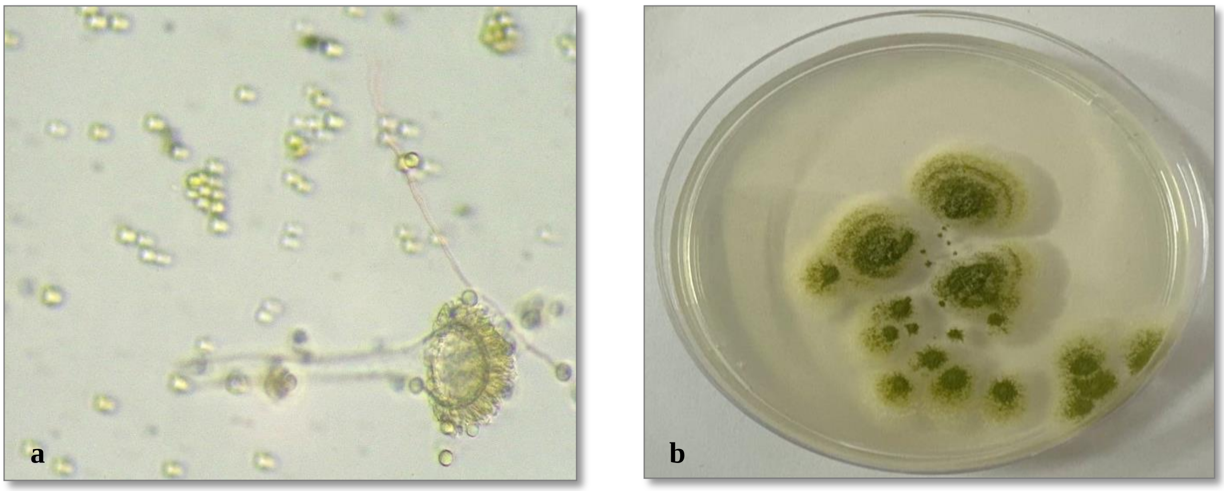

Figure 1. Shows the fungus *Aspergillus flavus* at 7 days and temperature of 25±2°C growth on PDA medium, a. microscope, b. colony.

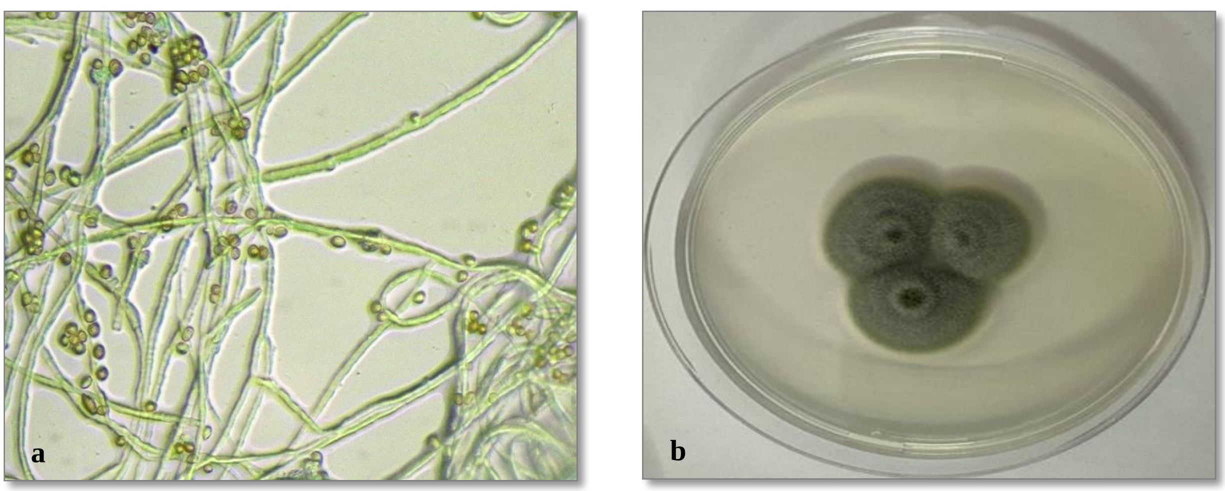

Figure 2. Shows *Cladosporium* sp. fungus at 7 days and temperature of 25± 2°C growth on PDA medium. a. microscope, b. colony.

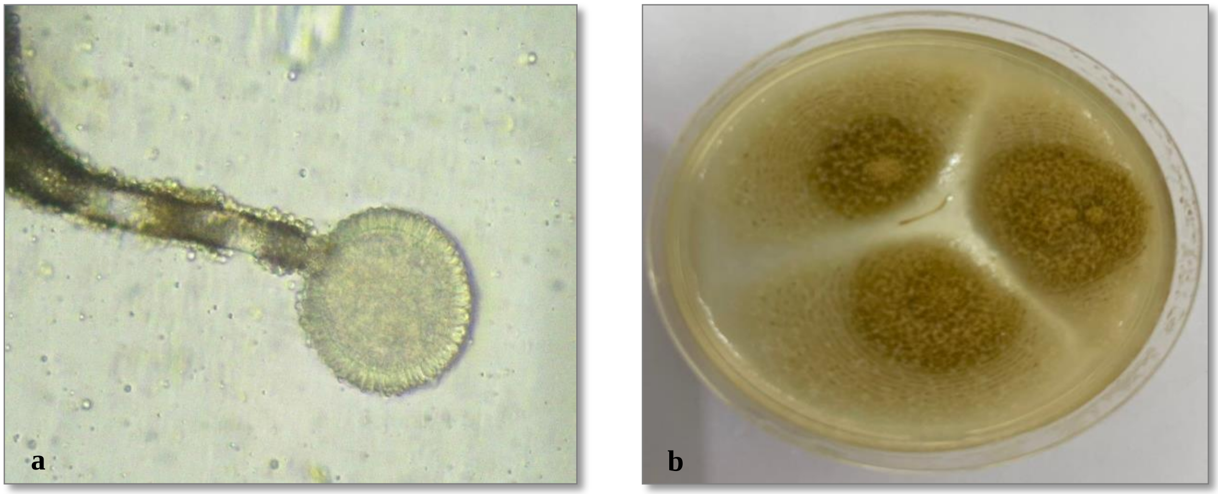

Figure 3. Shows the fungus *Aspergillus ochraceus* at 7 days and temperature of 25±2°C growth on PDA medium. a. Microscope, b. Colony.

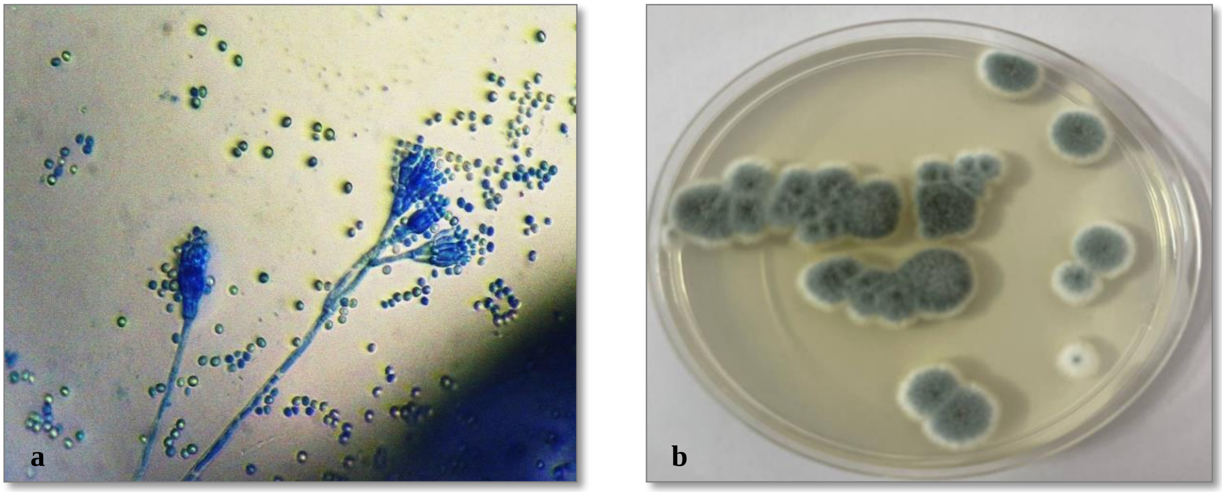

Figure 4. Shows the fungus *Pencillium griseofulvum* at 7 days and temperature of 25±2°C growth on PDA medium. a. Microscope b. Colony.

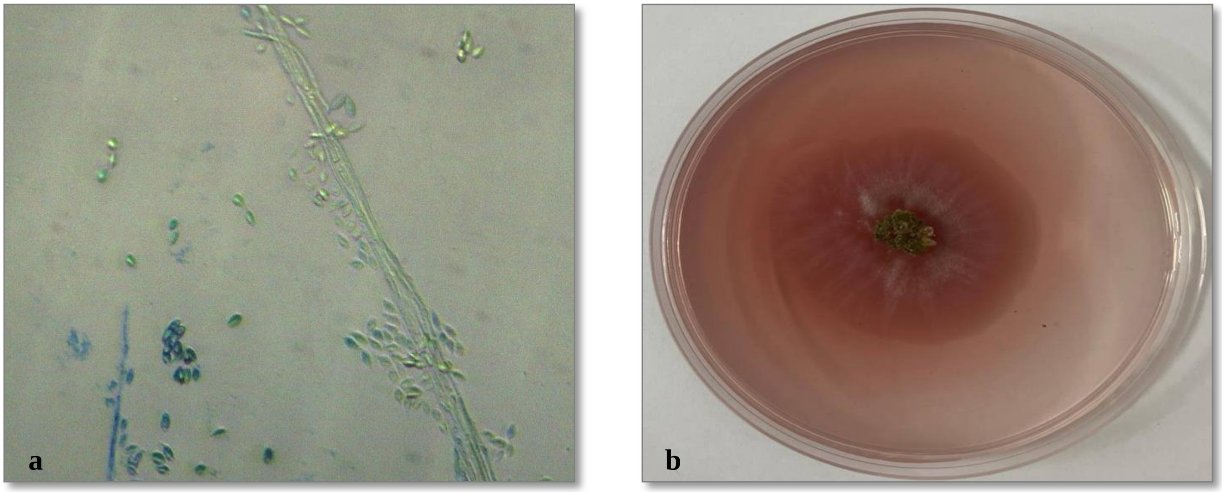

Figure 5. Shows the fungus *Fusarium solani* at 7 days and temperature of 25±2°C growth on PDA medium. a. Microscope, b. Colony.

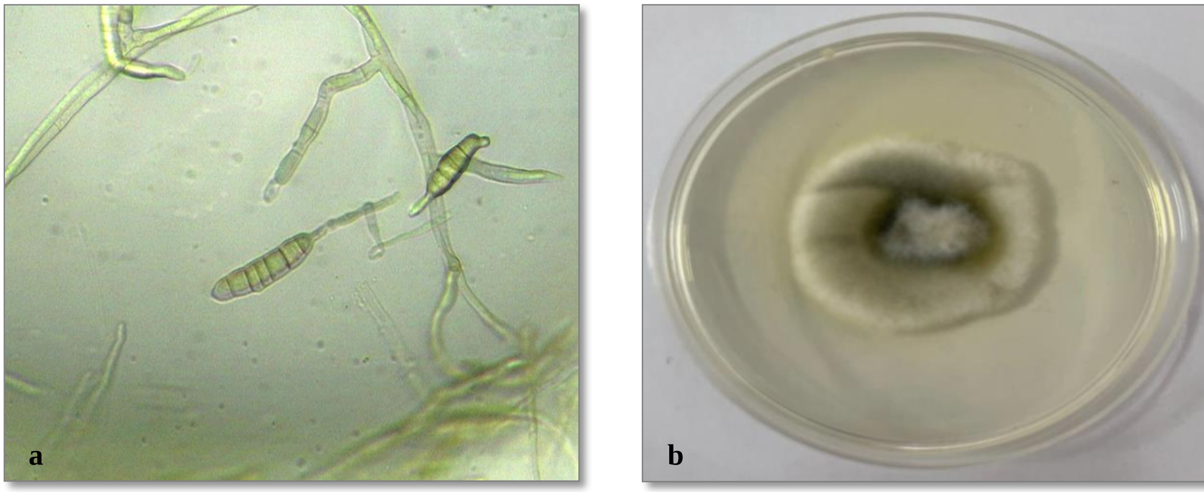

Figure 6. Shows the fungus *Altrenaria alternata* at 7 days and temperature of $25\pm2^{\circ}$C growth on PDA medium. a. Microscope b. Colony.

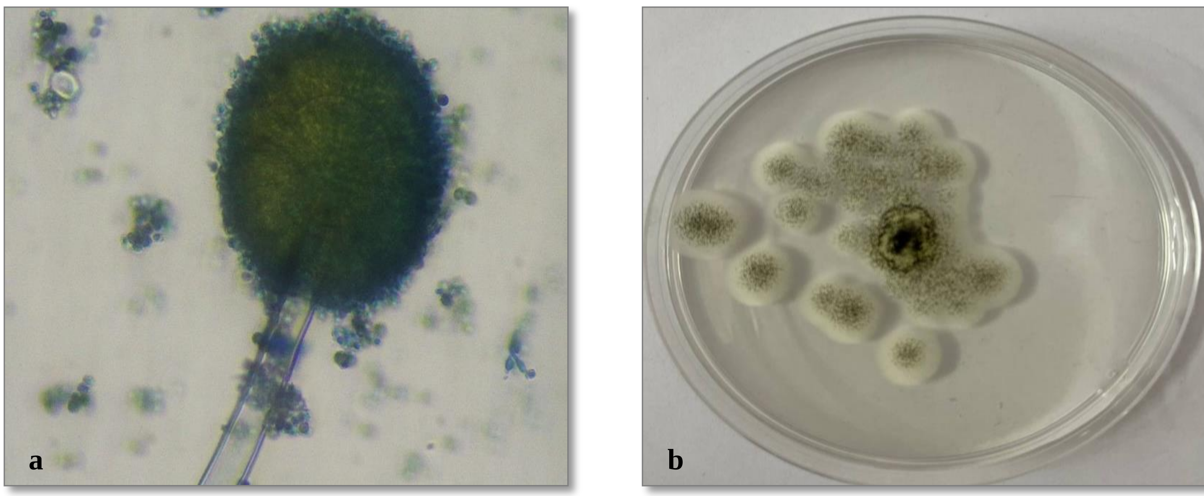

Figure 7. Shows the fungus *Aspergillus niger* at 7 days and temperature of $25\pm2^{\circ}$C growth on PDA medium. a. Microscope, b colony.

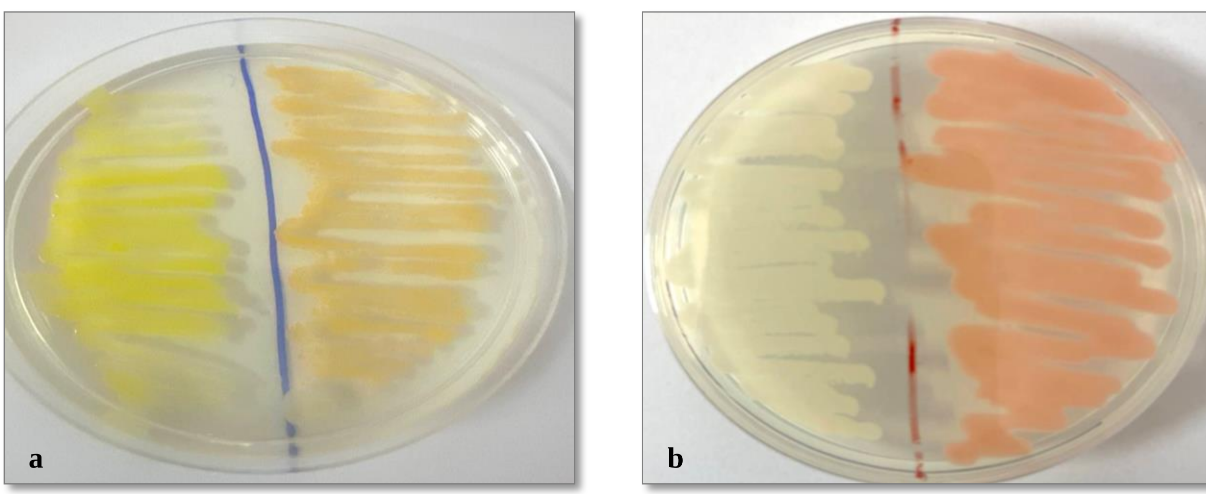

Figure 8. Shows the fungus color yeast at 7 days and temperature of $25\pm2^{\circ}$C growth on PDA medium.

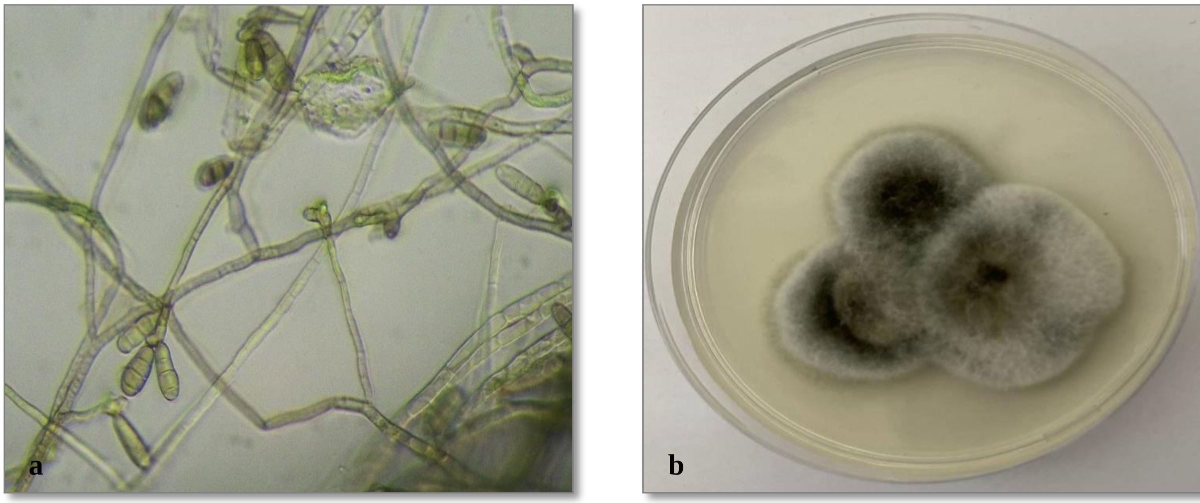

Figure 9. Shows the fungus *Curvularia* sp. at 7 days and temperature of $25\pm2^{\circ}$C growth on PDA medium.
a. Microscope, b. Colony.

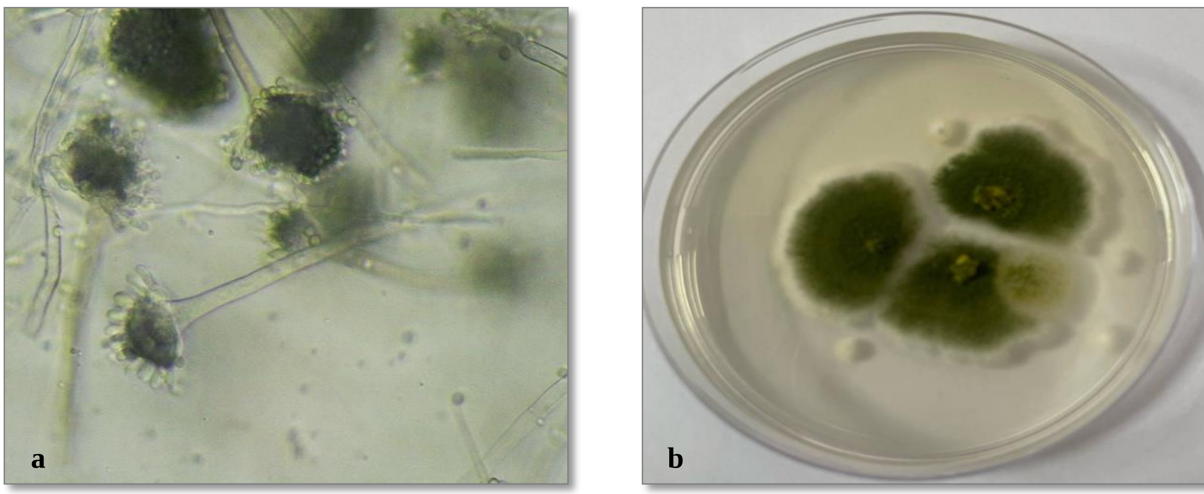

Figure 10. Shows the fungus *Aspergillus nidulans* at 7 days and temperature of $25\pm2^{\circ}$C growth on PDA medium.
a. Microscope, b.Colony.

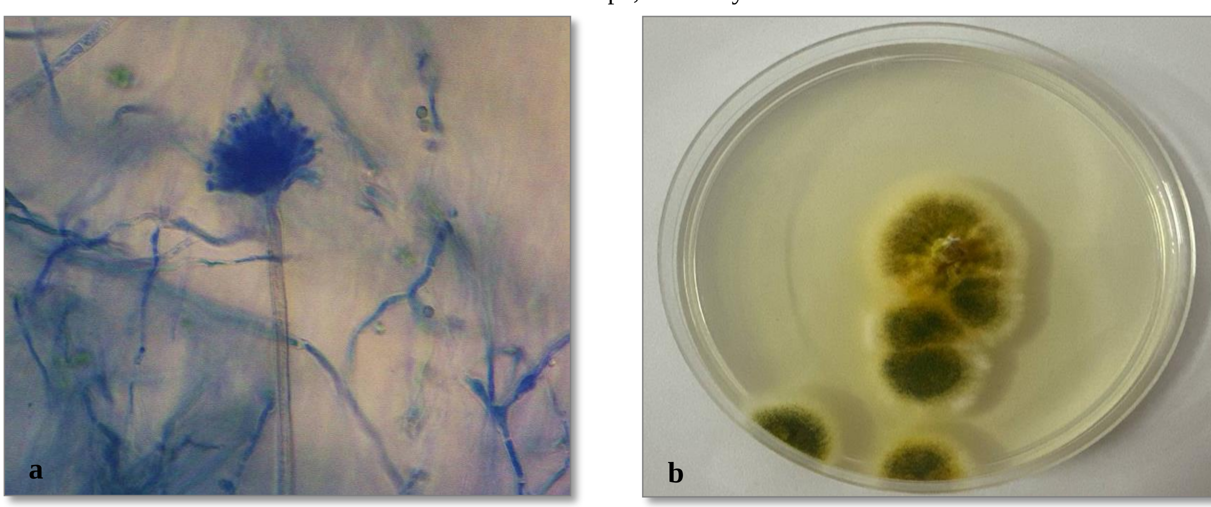

Figure 11. Shows the fungus *Aspergillus caespitous* at 7 days and temperature of $25\pm2^{\circ}$C growth on PDA medium.
a. Microscope b. Colony.

4. Discussion

The variety of fungal species related to petroleum-impacted soils indicates ecological adaptation to hydrocarbon stress. The prevalence of Aspergillus and Penicillium species is consistent with previous reports from similar sites in Basrah[7] and Trinidad[11], indicating an adaptive potential for tolerating and enzymatically degrading hydrocarbons. The fungus *Aspergillus flavus* was the most frequent and prominent among all isolates. Colonies of the fungus appeared in a light green color with white borders and a moderate growth rate[23], it is a fungus that usually exists in the soil; hypha contains septa. The fungus *Cladosporium* sp. affects plants and is often isolated from the soil, and it can cause allergic diseases in humans and animals. This fungus is characterized by its black or dark olive green color, and there are no white borders at the edge of the colony[24]. The fungus *Aspergillus ochraceus* appears with a pale yellow or yellow-brown color and shows noticeable rapid growth with a flaky texture. It is a widely distributed fungus isolated from several sources, such as soil, and it is a pathogenic fungus responsible for producing the most important mycotoxins, ochratoxins, penicillic acid, dihydropenicillic acid, and viomellein. These compounds demonstrate promising biological activity; they are antimicrobial, cytotoxic, antiviral, anti-inflammatory, antioxidant, neuroprotective against Parkinson's disease, and insecticidal[25]. The fungus *Pencillum grisofulvum* fungus is characterized by its green to gray color with white margins, causing post-harvest diseases and blue mold in stored fruits[26]. While the fungus *Fusarium solani* is characterized by its bright pink color, which is one of its vibrant colors. The colony of *Alternaria alternata* fungus appears black, and over the days, the upper surface of the colony starts to change to a gray-white color[27]. As for the white yeast *Candida* spp. is characterized by black colonies with white edges and has a very rapid growth, isolating this fungus from soil, air, surfaces, planes, homes, and hospitals, and it causes diseases in plants and humans[28]. The colonies of fungus *Curvularia* sp. start white to gray and then gradually darken until they reach a blackish-brown color. The colony morphology is velvety and it is a pathogen for plants, especially rice leaves, and is isolated from the soil[29]. The fungus *Aspergillus nidulans* is characterized by colonies with a wool-like texture and a distinct olive green color, and it is greatly affected by any changes in temperature. It is isolated from soils and grains and is an opportunistic fungus that infects patients suffering from immune deficiency[30]. Whereas the colonies of *Aspergillus caespitous* appear yellowish-green with dark green spores and slow growth. The fungus *Rhizopus stolonifer* exhibits dense cotton-like growth and rapid growth compared to other non-septate hyphae species, in addition to the appearance of sporulation and root-like structures above the medium. On the other hand, *Fusarium oxysporum* is characterized by its white color and septate hyphae.

These fungi are capable of producing oxidative enzymes (laccases, peroxidases, and monooxygenases) that degrade aromatic hydrocarbons. Their presence at oil contaminated sites indicates a possible enzymatic role in the assimilation and detoxification of hydrocarbons[31].

At last, the types of fungi varied with respect to the sampling sites. For example, *Aspergillus flavus* and *Candida* spp. were present in the same two locations, whereas *Curvularia* sp. and *Rhizopus stolonifer* were more prevalent in the agricultural soils, which may reflect differing availabilities of nutrients and pollution stresses[32].

5. Conclusion

The study identified a rich community of fungi associated with petroleum-contaminated soils in Iraq, with a majority of strains belonging to the phylum Ascomycota, and the highest presence of *Aspergillus flavus*. The fungal isolates displayed a diverse range of morphological adaptations and enzyme capabilities that led to a consistent degradation of hydrocarbons. These results illustrate the potential use of native fungi in bioremediation of petroleum-contaminated sites in the future.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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