International Science and Technology Journal
المجلة الدولية للعلوم والتقنية
Volume 38 العدد
Part 2 المجلد
المجلة الدولية للعلوم والتقنية
International Science and Technology Journal
ISTJ

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http://www.doi.org/10.62341/istj-vol38-2-irego14

Received	**2026/05/21**	**تم استلام الورقة العلمية في**
Accepted	**2026/06/09**	**تم قبول الورقة العلمية في**
Published	**2026/06/11**	**تم نشر الورقة العلمية في**

Evaluation of the efficiency of certain fungal strains isolated from oil-contaminated soil in the degradation of crude oil extracted from the Tubesti field, Libya

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Abstract

This study aimed to isolate fungal strains from oil-contaminated soil in Libyan oil fields and from selected car workshops and oil-change centres, to evaluate their potential in the biodegradation of crude oil, and to determine their hydrocarbon-degradation capacity. Six fungal strains were identified and isolated: *Aspergillus fumigatus*, *Aspergillus clavatus*, *Aspergillus terreus*, *Aspergillus oryzae*, *Penicillium sp1* and *Penicillium sp2*. The growth performance of these isolates was tested on Potato Dextrose Agar (PDA) medium supplemented with different concentrations of crude oil (1%, 3% and 5% v/v) *A.clavatus* exhibited the highest growth diameter, whilst *A. terreus* exhibited the lowest growth diameter at all concentrations. Biodegradation performance was also measured using the gravimetric method, and oil degradation rates at concentrations of 1%, 3% and 5% in the medium were: 90%, 78% and 60%, respectively, when using *A. fumigatus*. In contrast, *Penicillium sp1*, which recorded the lowest consumption rates; this is attributed to the inhibition of microbial enzyme activity at high

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contamination levels. The results demonstrate the efficacy of local Aspergillus isolates as promising candidates for the remediation of oil-contaminated areas.

This scientific manuscript was presented at the sessions of the International Renewable Energy, Gas, Oil and Climate Change Conference "iREGO" in the period of April 25-27, 2026. Tripoli - Libya

Keywords: Crude Oil; Tibesti Field; Aspergillus; Penicillium; Hydrocarbon Degradation.

تقييم كفاءة بعض السلالات الفطرية المعزولة من ترب ملوثة بالنفط على تحلل النفط الخام المنتج من حقل تبستي– ليبيا

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ملخص

أجريت هذه الدراسة لتقييم كفاءة بعض السلالات الفطرية المعزولة من ترب ملوثة بالنفط ومشتقاته من أماكن مختلفة، وهي ورش صيانة السيارات وأماكن تغيير الزيوت، وتحديد قدرتها على تحلل النفط الخام المستخرج من حقل تبستي، حيث تم عزل ست سلالات فطرية وهي *Aspergillus fumigatus* و *Aspergillus clavatus* و *Aspergillus terreus* و *Aspergillus oryzae* و *Penicillium sp1* و *Penicillium sp2*. تم اختبار نموها على وسط Potato Dextrose Agar (PDA) المعدل بنسب مختلفة من تركيز النفط الخام (1٪، 3٪ و 5٪، حجم/حجم)، وأظهرت *A.clavatus* أعلى قطر نمو على سطح الأجار الصلب، بينما أظهرت *A. terreus* أدنى قطر نمو في جميع التركيزات. كما تم قياس أداء التحلل البيولوجي بطريقة قياس الكتلة، وكانت معدلات تحلل الزيت بتركيزات 1 و3 و5% في الوسط: أعلاها عند استخدام

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Aspergillus fumigatus(90 و78 و60%) على التوالي. وعلى العكس من ذلك، سجلت *Penicillium sp1.* أدنى معدلات استهلاك، علاوة على ذلك، وجدت الدراسة انخفاضًا ملحوظًا في نسبة التحلل مع زيادة تركيز الهيدروكربونات، وهو ما يُعزى إلى تثبيط نشاط الإنزيمات الميكروبية عند مستويات التلوث المرتفعة.

وقد تم عرض هذه الورقة العلمية في جلسات المؤتمر الدولي للطاقة المتجددة والنفط والغاز وتغير المناخ "أيريقو" في الفترة 25-27 ابريل 2026م. طرابلس – ليبيا

الكلمات المفتاحية :المعالجة البيولوجية; النفط الخام ; حقل تبستي، Aspergillus ; Penicillium ; تحلل الهيدروكربونات.

1. Introduction

An oil substance, known as crude oil, contains hydrocarbons as well as various other chemical substances, such as organic oxygen, nitrogen, or sulfur compounds, which may be toxic to life at high enough concentrations [1]. Examples are some compounds present in crude oil that have hazardous and carcinogenic hydrocarbon properties [2]. The increase in consumption of oil combined with the oil and gas industry lead to negative environmental impact through oil products created as a result of crude oil extraction and transformation, leading to environmental pollution due to significant oil infrastructure failures [3].

Microbes can break down pollutants; this is called bioremediation. This method is widely used in the oil industry to clean up spills, The process of cleaning oil spills using bioremediation has many advantages. These include ease of use, ease of maintaining, and being able to use it over a large area to achieve total cleanup [4]. Hydrocarbon compounds in the environment can be transported in the ecosystem to both animals and plants, and they can cause toxicity to both plants and animals through threats of cancer and mutations. Hydrocarbon contamination has negatively impacted the microbial communities

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present in soil [5]. An example of this is in that gasoline contamination has poisoned many soil microorganisms [6]. The ability of fungi to contribute to bioremediation is due to their ability to grow rapidly and produce large quantities of biomass along with their ability to form long, extensive hyphae into the surrounding environment [7]. There are many fungi that have been isolated from the environment of oil spills that can successfully degrade oil through bioremediation [8].

Within this context, there is substantial interest in the use of fungi as bioremediation agents for the remediation of hydrocarbon-contaminated sites by utilizing fungi for the secretion of enzymes, which are subsequently used to degrade or remove hydrocarbons from the environment [9].

Fungi have been shown to play a major role in the degradation/removal of harmful compounds from soils and waters contaminated with oil products, as they naturally occur on such substrates and utilise hydrocarbons as a carbon and energy source [10].

2.Materials and methods

2.1 Collection of Sample

2.1.1 Soil samples

Soil samples contaminated with hydrocarbons were collected in substantial quantities. The first set of four samples was obtained from various locations around an oil-change establishment in the Brack area; the next four were collected from locations near auto repair workshops; and the remaining soil samples were taken from areas surrounding oil wells in the Tibesti oil fields. Sampling sites were selected based on visible contamination by crude oil and/or its derivatives, and all samples were collected from a depth of 5–15 cm below the soil surface [11].

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1.1.Oil samples

Crude oil samples were collected from Tibesti field, in opaque bottles, transported to the laboratory, and preserved until use. After that different concentrations were used in biodegradation experiments.

2.3. Preparation nutrient media:

The isolation and cultivation processes were performed using Potato Dextrose Agar and Czapek's agar. Following the manufacturer's guidelines, 39 grams of the particular medium were measured and incorporated into one liter of distilled water in a glass flask. Proper homogenization was ensured by vigorously shaking the nutrient media mixture and then heating it for 15 minutes on an electric shaker. [12].

Isolation of Fungi from soil contaminated by the Dilution method: (three dilutions for each soil), where 1 gram of contaminated soil with hydrocarbon was taken and placed in a test tube containing 9 ml of sterile distilled water, and was shaken for 5 minutes to represent the first dilution (10^{-1}), and 1 ml from the first dilution was placed in 9 ml of distilled water to represent the second dilution (10^{-2}), and 1 ml from the second dilution was taken and placed in 9 ml of distilled water to represent the third dilution (10^{-3}) All types of soil were used in the same way. 1 ml and 0.1 ml of each dilution (separately) were taken using a sterile pipette, and distributed on the plates prepared with the nutrient medium using a diffusion method. Three different nutrient media were used to determine which was best growth for fungi. The dishes were incubated at a temperature of 28°C, with three replicates for each treatment [13].

The direct soil scattering technique was used to isolate fungi. One gram of contaminated soil was scattered onto pre-prepared nutrient media in 9-cm Petri dishes and incubated at 28 °C in

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triplicate [12]. Following incubation for 3 to 5 days, fungal mycelia were subsequently subculture onto fresh media in order to isolate pure strains. Daily visual records were kept during this secondary incubation phase [14]. Microscopic identification was subsequently performed by preparing slide mounts from the growing colonies to assess the size, shape, and arrangement of conidia, using standard taxonomic keys as references [15,16]. Testing the efficiency of isolated fungi on decomposing hydrocarbon compounds.

The hydrocarbon-degrading efficiency of the isolated fungi was evaluated by monitoring their growth on media containing 1%, 3%, and 5% crude oil. Test plates were inoculated centrally with 5-mm mycelial plugs taken from active PDA cultures, then incubated at 28 °C for one week. Finally, the radial growth diameters of the resulting colonies were measured and compared with control plates to identify the most robust isolates [17].

2.4.Gravimetric method

Sterile liquid potato dextrose medium was prepared and dispensed into 150 ml bottles (50 ml per bottle). Crude oil at concentrations of 1%, 3%, and 5% (v/v), sterilised by filtration, was added to each bottle. Bottles were inoculated with 1 ml of each fungal isolate suspension and incubated in a shaking incubator at 28 °C for 15 days. The percentage of hydrocarbon consumption was then calculated as described below.

The residual hydrocarbons were quantified gravimetrically using pre-dried and pre-weighed sterile Whatman filter paper (No. 1). The culture medium was filtered through the paper, which was subsequently rinsed with diethyl ether to extract any remaining hydrocarbons. The filter paper was then placed in a drying oven at 45 °C for 24 hours to allow complete solvent evaporation. The mass

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of residual hydrocarbons was determined by calculating the difference in filter paper weight before and after filtration.

$$\textbf{Biodegradation\%} \frac{\text{sample hydrocarbons quantity} - \text{control hydrocarbons quantity}}{\text{control hydrocarbons quantity}} \text{ x } 100$$

The filter paper was reweighed to determine the mass difference before and after filtration, where the weight of the residue represents the remaining hydrocarbons. The entire procedure was carried out under sterile conditions [20].

3. Results and Discussion:

3.1. Isolation of fungi from soils contaminated with hydrocarbons

The results obtained after isolating 6 species of fungi, including *Pencillum sp* and *Aspergillus sp*. These findings are consistent with those reported in [1, 13, 21]

Treating fungal isolates with different concentrations of hydrocarbons from the results obtained and shown in Table (1) after treatment with the used concentrations of crude oil at the rates of 1%, 3%, and 5%, for a Tibesti field we find that , the largest diameter of colony at 1% *A. clavatus* 5.7 cm, A. fumigatus 4 cm, *Pencillum sp*$_1$ 4 cm, *A. oryzae* 3.2 cm, *Pencillum sp2* with a size of 2.9 cm, followed by *A.terreus* the small diameter 2.48cm.

In the case of 3%, we found that the highest diameter of colony for *A.fumigatus* and *A. clavatus* 4.35 cm for each, followed by A.oryzae, *Pencillum sp*$_2$ and *Pencillum sp*$_1$ with sizes growth of 3cm for each, while the lowest diameter of colony was *A.terreus* 2.56 cm.

As for the 5% treatment, the highest diameter of colony 4.43 cm for *A.fumigatus, A.clavatus* 4.25 cm and 4 cm for *Pencillum*

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sp₂, the lowest diameter of colony *A. terreus, A. oryzae* 2.14, 2.5 cm respectively.

Table (1): Fungal inhibition diameters on Tibesti oil field

FUNGI	Crude Oil Concentration	Average (cm)
A.Fumigatus	**Control**	7.23
	%1	4
	%3	4.35
	%5	4.43
Penicillium.Sp₁	**Control**	3.5
	%1	4
	%3	3
	%5	2.7
A.Terreus	**Control**	5.18
	%1	2.48
	%3	2.56
	%5	2.14
A.Clavatus	**Control**	3.8
	%1	5.7
	%3	4.35
	%5	4.25
Penicillium.sp₂	**Control**	2.2
	%1	2.9
	%3	3
	%5	4
A. Oryzae	**Control**	1.7
	%1	3.2
	%3	3
	%5	2.5

Results of hydrocarbon consumption by fungi using the gravimetric method

Different fungi were found to have different abilities to degrade crude petroleum (figure 1) based on the results of our gravimetric assay. The most effective fungus, *A. fumigatus*, had the highest rate of crude petroleum degradation at a concentration of 1% with a degradation rate of 90%. The same fungus was previously reported

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to be able to degrade hydrocarbons at a rate of 71.7% [3]. Other fungi with high degradation rates were *Penicillium sp₂* and *A. terreus*, which degraded 89% and 86% of the petroleum, respectively. Interestingly, *A. terreus* had the smallest colony diameter, which was previously related [18].

Aspergillus oryzae was noted to have an 85% degradation rate, and the results of this assay support the previously reported value of 90% bioremediation of soil-bound hydrocarbons by *A. oryzae* (9). Both *A. clavatus* and *Penicillium sp₁* were found to have 83% degradation rates of crude oil. These results are consistent with those of [4], who demonstrated the efficacy of these two oil-environmental isolates for enhancing the rate of removal of used motor oil from contaminated surfaces.

Increasing the concentration of crude oil from 1% to 3% inhibited the degradation of crude oil by the isolates tested. However, *A. fumigatus* continued to perform the best, with a 78% degradation of crude oil. Both *Penicillium sp₂* and *A. clavatus* also exhibited significant tolerance with a degradation of 76% and 73%, respectively.

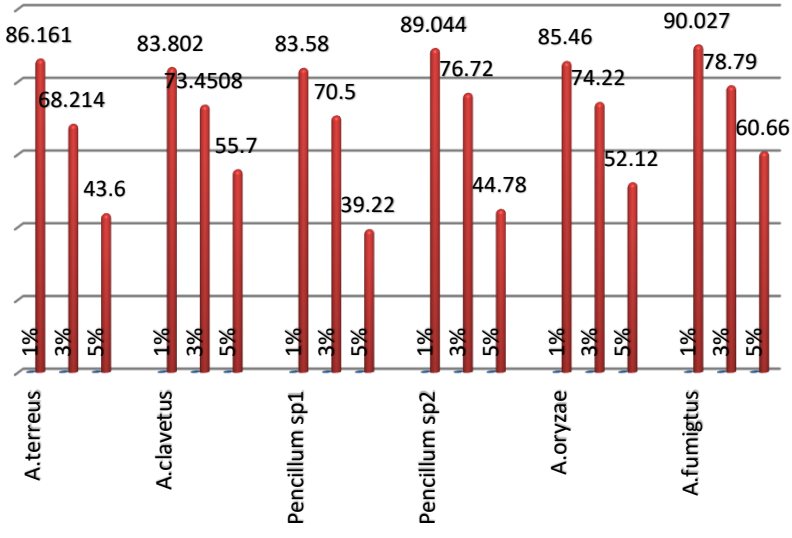

Figure 1: Percentage of fungal consumption of crude oil

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When treated with a concentration of 5%, the highest consumption rate was for *A.fumigatus*, which was 60%, followed by *A.clavatus*, with a rate of 55%, A.oryzae, with a rate of 52%, and Pencillum sp2, with a rate of 44%, and the lowest percentage was for the two fungi, *A. terreus* and *Pencillum* sp_1, which is 43% and 39%, respectively. From the above, we find that the highest percentage of consumption of all concentrations was by the fungus *A.fumigatus*, the lowest percentage of consumption by *Pencillum* sp_1. The results also showed that the percentage of consumption decreases as the concentration of hydrocarbons increases, and this is consistent with the study [1] Also, all results showed that an increase in the concentration of crude oil in the soil sample is associated with a decrease in the percentage of removal of hydrocarbons by *Pencillum* sp., *Aspergillus* sp., because hydrocarbon pollutants work to suppress the enzymatic activity of microbes when the percentage of pollution increases [21] and this is confirmed by the results obtained in this study.

From the results obtained in this study, we find that the presence of Aspergillus species was dominant compared to the presence of the Pencillum fungus variety, and that the dominance of Aspergillus fungi is consistent with what was found [12].

When comparing the results obtained in this study for Aspergillus species in terms diameter of colony, we find that it is higher than *A.fumigatus* and lowest for *A.terreus* in terms diameter of colony.

The presence of *A. terreus* through the results of this study is consistent with some studies, including to identify the dominant fungi in soil contaminated with oil and its derivatives, as well as [13]

Also, the presence of *A. oryzae* and the efficiency of its decomposition, as stated in [17] to describe the original fungi responsible for the decomposition of crude oil from the coastal area near the Red Sea in Yanbu, Saudi Arabia, and isolated from areas contaminated with oil. The fungi were also isolated. Aspergillus, Pencillum showed that *A. oryzae* more efficiently decomposes 99%

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of crude oil, while the consumption rate in the current study was 85% at a concentration of 1% of crude oil.

Conclusions

This study demonstrates the capability of indigenous soil fungi to serve as effective agents in the bioremediation of petroleum hydrocarbons. Among the isolates examined, Aspergillus fumigatus exhibited the highest hydrocarbon utilisation efficiency across all tested concentrations. In contrast, Penicillium sp1 recorded the lowest degradation rates, a finding attributed to inhibition of microbial enzymatic activity at elevated hydrocarbon concentrations. The remaining fungal isolates displayed intermediate degradation efficiencies, underscoring the potential of locally isolated Aspergillus strains as promising candidates for bioremediation applications in oil-contaminated environments.

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