



# Biodegradation of oil-contaminated soils by *Aspergillus Niger* and *Aspergillus Flavus*

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**ABSTRACT.** The use of microorganisms is one of the most important methods widely used in the world to treat hydrocarbon pollution by decomposing them. A nutrient medium with mineral salts and added crude oil was used to grow fungi isolated from contaminated sites, namely *Aspergillus flavus* and *Aspergillus niger*. During the incubation period of 20 and 40 days, gas chromatography (TPH) was used to measure hydrocarbons. Over a similar period of time, the results revealed that *Aspergillus flavus* performed less well in decomposition (32.5% and 60.2%) against *Aspergillus niger*, which recorded higher decomposition rates (44.5% and 74.6%). This leads to the fact that fungi, through their enzymes, are able to analyze fungal pollutants in the soil.

**Keywords:** Degradation; TPH; fungi; bioremediation; gas chromatography.

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## Introduction

Environmental pollution resulting from industrial human activities is one of the most dangerous types of pollution that affects ecosystems through the toxic elements it produces (Znad et al., 2021). Human activity of exploring, extracting and transporting crude oil has cast a shadow over ecosystems through the hydrocarbons it emits, which are among the most important and dangerous pollutants when they reach ecosystems, such as soil and water, and which are difficult to decompose spontaneously (Varjani, 2017). Treating hydrocarbon pollutants with chemicals or preparing sites to bury them leads to more environmental pollution and is very expensive. This is what prompted scientists to invent less expensive treatment methods that are more sustainable for the ecosystem and less harmful, which is treatment using microorganisms and fungi, for example, by using their enzymes to decompose (Das & Chandran, 2011).

The composition of crude oil consists of complex compositions of hydrocarbons and non-hydrocarbons, in addition to other heavy elements such as nickel and copper (Santos et al., 2014). Hydrocarbons, by their nature, are distinguished into aliphatic and aromatic varieties (Sari & Trihadiningrum, 2018).

One of the main components of crude oil is petroleum hydrocarbons (TPH), which are formed from hydrogen and carbon and make up about 55% to 98% of oil (Al-Dhabaan, 2019).

Crude oil has become an essential component in many industries such as fertilizers, asphalt, etc Which, in one way or another, emit pollutants that cause damage to the ecosystem (Brown et al., 2017; Marchand et al., 2017).

One of the most important global environmental problems that requires an urgent solution is soils contaminated with crude oil and the heavy elements it contains, such as lead and cadmium (Azaizeh et al., 2010; Kistic et al., 2010).

Using traditional methods to treat this pollution also produces pollutants and is very expensive, which makes it ineffective and unsustainable, which has made biological treatment of oil pollutants an alternative and urgent solution (Mohammadi-Sichani et al., 2017).

With their enzymes and ability to decompose oil pollutants, *Aspergillus* fungi are establishing themselves as the first solution to treat pollution (Harms et al., 2011). After studying all the genera of *Aspergillus*, two important genera were found: *Aspergillus niger* and *Aspergillus flavus*, which showed a high ability to break down petroleum compounds (Okigbo et al., 2015; Khan et al., 2020).

The process of (bioremediation) began to spread rapidly after traditional treatments were unable to solve the problem of pollution by oil vehicles, as it was distinguished by the fact that its microorganisms used the carbon found in oil vehicles as their energy source (Shraddha et al., 2011).

With its efficient metabolic system via its hydrolytic enzymes, microorganisms have been used to break down hydrocarbon compounds and convert them into minerals used in building their bodies (Dahan et al., 2017; Zekri & Chaalal, 2005). This has made it receive increasing global attention for its importance, the cost of its use, and the fact that it does not cause harm to the ecosystem (Clarkson & Abubakar, 2015; Vidali, 2001; Thenmozhi et al., 2011). The use of biological treatment using microorganisms in treating contaminated soil is subject to several conditions and factors, including the availability of nutrients, acidity, soil type, and other physical properties (Aharoni et al., 2017; Avishai et al., 2017).

This research seeks to provide more efficient ideas and solutions through the use of microorganisms (fungi) in treating crude oil pollutants under controlled conditions

## Material and method

### Sample Oil

Southern Oil Company (Basra, Iraq) was responsible for transporting the oil sample. The sample was sent to North Oil Company (Nineveh, Iraq) with the cold and dark lab equipment until it was used. The same conditions were maintained until the work was finished. Crude oil was present in soil samples. Table 1 and Figure 1 present the soil samples obtained from three sites at Al-Qayar refinery in Mosul city, Iraq with a depth range of 0-15cm.

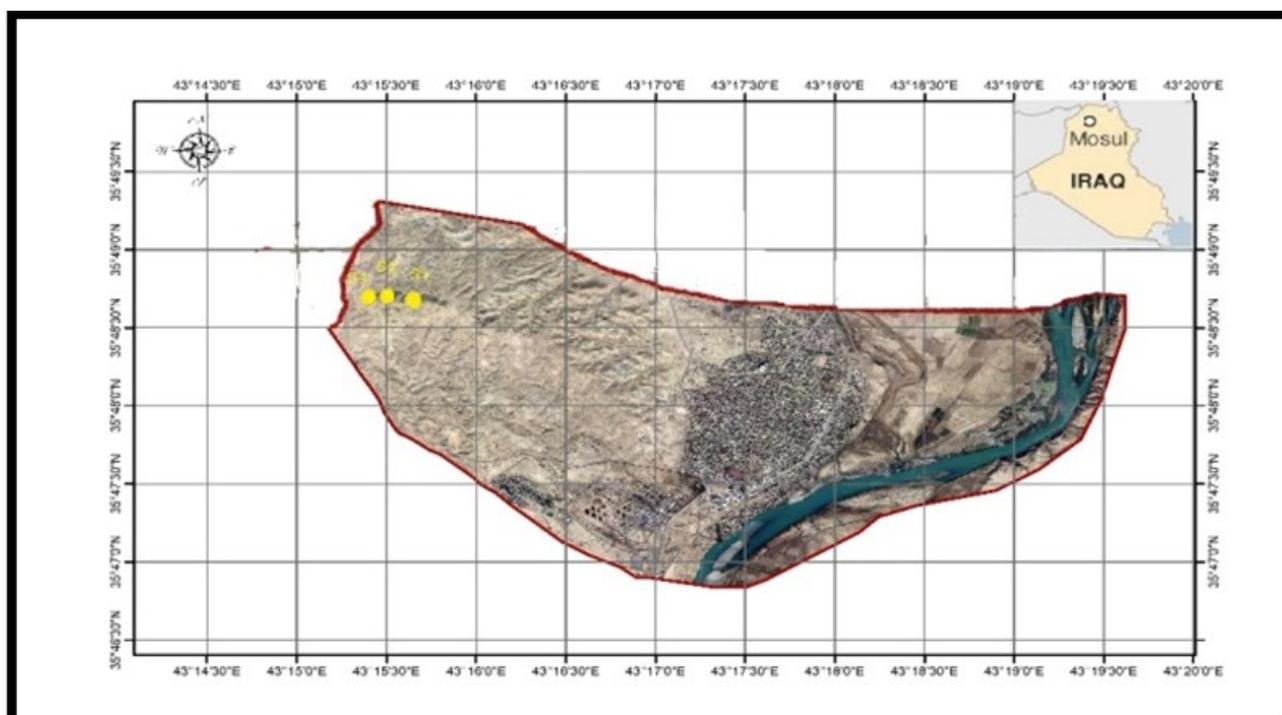


Figure 1. Shows the sites of taken samples.

Table 1. Shows the coordinates of collected samples.

Longitude	Latitude	No. of samples
43.2546	35.8127	S1
43.2573	35.8121	S2
43.2592	35.8134	S3

### Isolation and diagnosis of fungi

The dilution method was used to isolate the fungi species and the polymerase chain reaction (PCR) technique was used to diagnose them. To achieve the desired dilution ratio of 10<sup>-1</sup>, a contaminated soil sample was soaked in 9 ml of sterile distilled water and crude oil during the procedure. Petri dishes containing 10 ml of PDA medium were used to plant 1 ml of the diluted solution. Meysami & Baheri (2003) incubated the dishes at 28°C for 7 days.

By using a light microscope and PCR technique, the fungal colonies that were the most abundant and abundant in Petri dishes were isolated and diagnosed for the purpose of degrading TPH compounds.

### Growth of fungi used in the study

Mineral salts medium (MSM) prepared according to (Zajic & Supplisson, 1972) in order to use in growing isolated fungi species selected for the degradation processes. The pH of the culture medium was adjusted at 5.3, then sterilized and transferred 50ml of sterilized medium to the conical flask 250 ml. The crude oil was added to the medium with a concentration of 5% (v/v). Then 2 ml from the spore suspension of *Aspergillus Niger* and *Aspergillus Flavus* (every 1 ml of spore suspension containing  $2.1 \times 10^6$  spore/ml) were added to the culture medium. Finally, the conical flasks were incubated at 28°C for 20 and 40 days. On the other hand, the control flask was incubated at the same time with similar conditions but without adding spore suspension.

### Extraction of crude oil

Some modifications were made to the Mittal and Singh method for extracting crude oil from the liquid medium (Mittal & Singh, 2009) 50 ml of the liquid medium added to 50 ml of hexane in the separating funnel (1:1). The contents were mixed well in the separating funnel, then it was left for (5-10) minutes to release the gases from the valve. The mixture stabilized in the separating funnel so that, two layers were formed, the upper layer represents crude oil, whereas the lower layer represents (water + Hexane). The remaining moisture from crude oil was removed by passing it through a column that contained cotton and sodium sulfate anhydrous, then left overnight to evaporate more moisture. The same procedure was applied to the control flask under the same conditions.

### Samples measurement

The samples were analyzed using a gas chromatography device manufactured by Shimadzu company in Japan. The percentage degradation of the crude oil was calculated based on the following (Oudot, 1984).

$$\text{Degradation \%} = ((\text{mg of crude oil control} - \text{mg of crude oil test}) / \text{mg of crude oil control}) \times 100$$

### Statistical analysis

Statistical analyses were conducted using SPSS Independent t-tests were used to compare between two Fungal Species.

## Results and discussion

The two species of *Aspergillus sp.* have been diagnosed by PCR technique. *Aspergillus Niger* and *Aspergillus Flavus* demonstrated good growth in (MSM) contaminated with crude oil.

To assess the significance of the differences in total petroleum hydrocarbon (TPH) degradation between *Aspergillus niger* and *Aspergillus flavus*, a statistical analysis was conducted based on replicated experimental data (n = 3). The results revealed that *A. niger* exhibited a significantly higher degradation rate than *A. flavus* at both 20 and 40 days of incubation.

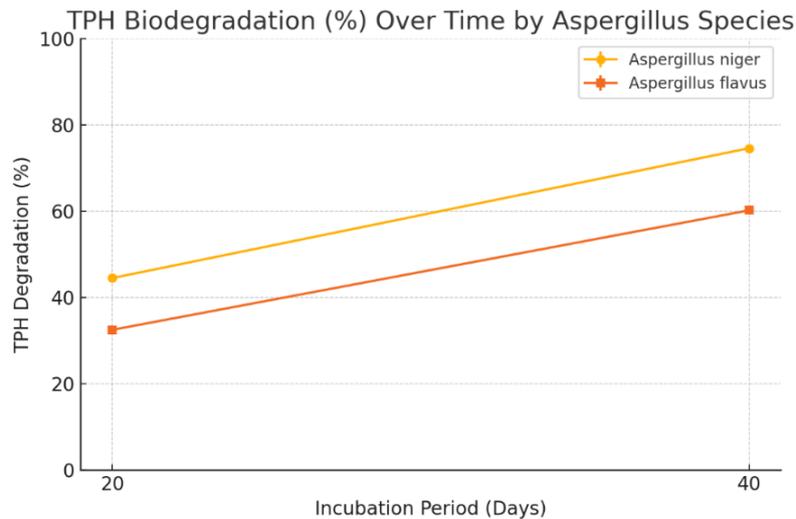
At 20 days, *A. niger* achieved an average degradation of  $44.5\% \pm 0.98\%$ , while *A. flavus* reached  $32.5\% \pm 0.76\%$ . After 40 days, *A. niger*'s degradation rate increased to  $74.6\% \pm 0.45\%$ , compared to  $60.2\% \pm 0.57\%$  for *A. flavus*. Independent sample t-tests confirmed that the differences in degradation efficiency between the two fungal species were statistically significant ( $p < 0.01$  at 20 days;  $p < 0.001$  at 40 days). As shown in Figure 2.

*Aspergillus sp.* is widely spread and adapted to grow in soils contaminated with petroleum hydrocarbons because they have special enzyme systems Which helps it break down and dismantle carbon compounds, as it can convert them into energy to carry out its life actions such as: Lignin, Manganese, Peroxidase (Durairaj et al., 2016).

The present study results show that there are differences between the *Aspergillus Niger* and *Aspergillus Flavus* in their ability to break down hydrocarbons. This indicates that biodegradation of hydrocarbon compounds depends on the type of microorganism, the incubation conditions, and the type of crude oil (Abioye et al., 2013) This study corresponds to the study by (Zhang et al., 2016) which isolated six species of fungi belonging to *Aspergillus sp.* and used it for bioremediation of soils contaminated with crude oil.

The results of this study differ from the study by (Maté et al., 2011) in which they found during their study the ability number of fungal species belonging to the *Aspergillus sp.* in the breakdown of petroleum

hydrocarbons, they notice that *Aspergillus Niger* has a weak ability to break down crude oil compounds due to its weak enzymatic ability to break down hydrocarbons.



**Figure2:** Comparison of TPH degradation (%) between *Aspergillus niger* and *Aspergillus flavus* over two incubation periods. Error bars represent standard deviation (n = 3).

The incubation period effect positively on the decomposition percentage of hydrocarbons, as increasing the incubation period raised the degradation percentage of hydrocarbon by *Aspergillus Niger* from 44.5% at 20 days to 74.6% at 40 days whereas raised from 32.5% at 20 days to 60.2% at 40 days by *Aspergillus Flavus*, these results are agreement with (Al-Jawhari, 2014) who mentions that the degradation percentage of crude oil hydrocarbons increases as the incubation period increases.

## Conclusion

The results of this study showed that bioremediation using fungi can be a promising and sustainable solution, after *Aspergillus niger* and *Aspergillus flavus* showed a high ability to degrade and decompose hydrocarbon pollutants, specifically *Aspergillus niger*, which recorded higher performance after long incubation conditions.

The proportions achieved by *A. niger* after a period of 40 days were striking and inspiring through its resistance and tolerance to harsh conditions in analyzing these pollutants and the ability of its metabolic system to work. This treatment can be widely used in treating many industrial pollutants.

This study recommends conducting further tests on these types of fungi and the extent to which the Targaba field is the reality that exists in these organisms in the sites from which they were isolated, and conducting a more in-depth economic feasibility study when using them in the same sites.

## Data availability

Data, numbers, statistics and research results are included in this article through tables and graphs

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