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Biodegradation Aromatic Hydrocarbons of Naphthalene by *Aspergillus flavus* and *Penicillium fumigates*

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ABSTRACT

Filamentous fungi were identified in the soil that had been contaminated by petroleum hydrocarbon and wastewaters released by the oil refinery of Nasiriyah in East part of the Nasiriyah city and Nasiriyah oil field (Al-Kati'a) by using dilution method. The study demonstrated a strong capability of *Aspergillus flavus* and *Penicillium fumigatus* to remove aromatic hydrocarbons, specifically Naphthalene. The biodegradation process was carried out under optimized conditions, using various concentrations of Naphthalene. The findings revealed that *Aspergillus flavus* and *Penicillium fumigatus* were the highest frequently isolated fungi, with *Aspergillus flavus* showing a 100% occurrence rate and *Penicillium fumigatus* 97%. Both fungi were exposed to petroleum hydrocarbons in both solid and liquid media and successfully demonstrated the capacity for degradation Naphthalene. Their colony diameters were measured on solid media, confirming their adaptability and growth in the presence of Naphthalene. Both fungi were capable of degrading Naphthalene at a concentration of 50 ppm. The degradation products and residual Naphthalene levels were identified using Fourier-transform infrared spectroscopy (FTIR) and gas chromatography-mass spectrometry (GC-MS). The results of statistical analysis showed that the two fungi's performance differed significantly.

Keywords: Biodegradation, Naphthalene, *A. flavus*, *P. fumigates,* GC-MS

INTRODUCTION

Petroleum hydrocarbon compounds are one of the main components of crude oil that affect the living and non-living components of the aquatic ecosystem (Batelle, 2000; Seiyaboh & Jackson, 2017). Oil, its derivatives and residues are among the most important elements and major pollutants in the aquatic environment, according to the definition, oil pollution occurs when an oil compound or one of its derivatives is introduced into the land or aquatic environment, which leads to damage through alteration in its physical, chemical, and biological characteristics, which consequently results in harm to humans either directly or indirectly (Al-Saad et al., 2003). Among the components of crude oil there is a group of compounds called Polycyclic Aromatic Hydrocarbons (PAHs) .It is a group of persistent and semi-volatile organic pollutants found everywhere in the environment and enter it through many sources such as industrial and agricultural pursuits (Wick et al., 2011; Ateshan & Misnan, 2025a). Oil pollution leads to a direct impact on human health, as it may affect respiration as a result of inhaling toxic gases resulting from the evaporation of crude oil, or these pollutants may cause birth defects and cancerous diseases due to direct exposure to carcinogenic pollutants such as Pyrene and Benzo[a] Pyrene, and oil residues from refineries may work when they reach the soil to directly affect living organisms (AL-Taher et al., 2020).

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Bioremediation is a technique which employs biological processes to either break down or neutralize pollutants, rendering them harmless (Azubuike et al., 2016; Al Sailawi et al., 2020). Compared to conventional methods, bioremediation is generally more cost-effective and can completely eliminate contaminants, providing a sustainable solution. It is also a minimally invasive approach that helps preserve the stability of ecosystems (Perelo, 2010; Ateshan & Misnan, 2025b). Bioremediation is particularly useful in situations where physical or chemical methods fail to effectively treat low concentrations of pollutants. For this process to work, microorganisms must use enzymes to degrade pollutants into non-toxic substances. The success of bioremediation largely depends on environmental factors that encourage microbial activity and growth. As a result, adjusting these environmental conditions is often essential to improve the efficiency of pollutant breakdown (Vidali, 2001; Jasim et al., 2021; Abdullah et al., 2022).

Recently, extensive research has been conducted on the ability of certain fungi isolated from refineries and oil fields to remove and degrade polycyclic aromatic compounds such as naphthalene, thus becoming a promising alternative to replace current treatment processes. This research focused on studying the ability of *Aspergillus flavus* and *Penicillium fumigates* to degrade naphthalene into simpler substances, and these two fungi were chosen because they are the most frequent fungi.

MATERIALS AND METHODS

Naphthalene Compound and Collection of Samples

Effluent and sediment samples gathered from the Nasiriyah oil refinery and the Nasiriyah oil field (Al-Kati'a). Fungal isolates were obtained from six different stations within Lab, with (3) replicate specimens which were taken from different places. In this study, naphthalene, a type of polycyclic aromatic hydrocarbon (PAH), was used. The naphthalene utilized in the experiments was sourced from BDH and Merck, with a chemical purity of 99.99%.

Fungal Isolation

Specimens acquired for fungal isolation was studied using the dilution method, used to fungi studied from sediment and water samples. Precisely, (1) ml of wastewater or (1) gram of dirt combined with (9) ml of Combined with distilled water and progressively diluted to a concentration of 10^{-4} (Al-Nasrawi, 2012; Ateshan et al., 2019; Ha et al., 2019). Following comprehensive mixing, the pour-plate technique was employed by transferring (1) ml from every dilution into sterile Petri dishes. Potato Dextrose Agar (PDA) medium, augmented with the antimicrobial drug chloramphenicol (250 mg), was then added. The plates underwent incubation at 25°C for a duration of seven days. Fungal growth was sub-cultured and examined under a dissecting microscope for further study. For fungal cultivation, a mineral salts medium was used, which contained (g/L): K_2HPO_4 (1.71), KH_2PO_4 (1.32), $NaNO_3$ (0.42), $MgSO_4 \cdot 7H_2O$ (0.42), and $CaCl_2$ (0.02). Fungal species were identified using general and specialized taxonomic references (Klich & Pitt, 1992; Ateshan & Saxena, 2015). In this study, naphthalene at concentrations of (50, 100, 150) ppm was used as the sole carbon and energy source.

The Capacity of Isolated Fungus to Proliferate on Solid Medium Enriched with Naphthalene

Fungal growth was assessed to identify isolates resistant to naphthalene on solid media, and the results were compared to a control. Naphthalene was incorporated into the warm Potato Dextrose Agar (PDA) at concentrations of 50, 100, and 150 ppm before the medium solidified in all experimental plates. Control plates were prepared without the addition of naphthalene. This experiment was conducted in duplicate. A 5 mm disc extracted from fungal colonies that

were 7 days old was used to inoculate each plate. The plates were then incubated at 25°C. After seven days, the colony diameters of all fungal isolates were measured and compared to those on the control plate (Al-Jawhari, 2016; Al-Husseini & Alsalman, 2019; Ateshan et al., 2020).

Isolated Fungi's Ability to Grow in Mineral Salt Medium Supplemented with Naphthalene

Fungal growth was also evaluated in liquid medium to determine the resistance of isolated fungi to naphthalene. Growth was compared to a control by measuring the mycelial biomass. A 250 ml volume of mineral salts medium, containing naphthalene at concentrations of 50, 100, and 150 ppm as the sole carbon source, was used. Five milliliter disc from seven-day - old fungal colonies were used to inoculate the liquid medium. Control flasks, which contained the same medium and naphthalene concentrations, were prepared without fungal inoculation. After sealing each flask with non-absorbent cotton wool, they were incubated for seven days at 25°C. During incubation, the flasks were agitated on an orbital shaker at 150 rpm to ensure proper mixing. After the incubation period, the fungal biomass was collected through the use of Whatman No.1 filter paper and its dry weight was measured using a sensitive balance. A pH meter was also used to record the culture medium's pH.

Biodegradation of Naphthalene

After seven days of incubation, gas chromatography-mass spectrometry (GC-MS) was used to determine the remaining naphthalene concentration. Naphthalene was extracted from the samples using hexane in a 1:2 ratios, followed by centrifugation at 10,000 g for 10 minutes. After phase separation, 1ml of the hexane layer was filtered through a 0.45 µm Millipore filter and transferred to a sterile vial for hexane evaporation. Once the hexane was completely evaporated, 1ml of acetonitrile was introduced to the remainder, and the residual naphthalene was examined via (FTIR) and GC-MS.

For the GC-MS analysis, a 1 µl sample was injected. After being initially set at 60°C and maintained for one minute, the oven's temperature was raised by 25°C every minute until it reached 150°C, followed by a rate of 10°C per minute up to 260°C, where it was held for 20 minutes. Finally, the temperature was raised to 270°C and maintained for another 20 minutes. The carrier gas, helium, flowed at a rate of one milliliter per minute. Simultaneously, residual naphthalene was also confirmed using FTIR analysis.

Statistical Analysis

The SPSS (version 23.0) package was used to analyze all applications using (ANOVA) in order to determine whether or not there was a significant difference.

RESULTS AND DISCUSSION

Isolation Fungi

Filamentous fungi were isolated from soil contaminated with petroleum hydrocarbons and from wastewater discharged by the Nasiriyah oil refinery, located in the eastern part of Nasiriyah city, as well as from the Nasiriyah oil field (Al-Kati'a). Table (1) presents the aggregate quantity of fungi genera identified in all selected, along with their frequency in both sediment and water. Table (2) indicates that (*Aspergillus flavus* and *Penicillium fumigatus*) were the highest commonly isolated fungi in this study, showing the highest frequency among all identified species. According to Table (2) the occurrence rate of *A. flavus* reached 100%, while *P. fumigatus* appeared with a frequency of 92%.

These findings differ from those reported by Al-Saidi (2015), who found the occurrence rates of *P. fumigatus* and *A. flavus* to be 69% and 78%, respectively, in samples from the

Nasiriyah oil refinery. However, the results of the present study are consistent with those of Al-Jawhari (2016), who also reported a 100% occurrence rate for both *A. flavus* and *P. fumigatus*.

The differences between sampling locations this study did not significantly influence fungal diversity inside the sediments, likely due to similar environmental conditions across all stations. Parameters including temp, DO, pH, and organic carbon were quantified. at each site to assess their influence on fungal distribution.

Table 1: Total number of fungal genera across all specimens, in conjunction with their prevalence in sediment samples

Fungi	Total number of genera across all samples	Frequency, %
Aspergillus	$10^4 \times 50$	53
Penicillium	$10^4 \times 45$	47

Note: Total number of all fungal genera 95×10^4

Table 2: Prevalence of species extracted from the upper layer of sediments

Kinds of fungi	The quantity of the fungal kinds is evident	Frequency, %
A. flavus	50	100
P. fumigates	45	97

Note: Number of samples studied = (50) samples

The Capacity of Secluded Fungus to Proliferate on Solid Substrate Enriched with Naphthalene

The results in Table (3) demonstrated that fungi proliferate effectively in PDA medium augmented with naphthalene, as evidenced by a daily augmentation in the diameters of isolated fungal colonies, signifying that these specimens may thrive in the presence of the aromatic compound. The study's results indicated that the average colony diameter of *A. flavus* during the fifth day of the experiment within the culture medium with naphthalene at concentrations of (50, 100, and 150) pm was (6.3, 7.5, and 8.3) cm, respectively. The diameter of the *A. flavus* control on the sixth day was (8.5) cm. The study's results indicated the mean diameter of *P. fumigatus* on (15) day of the experiment in the culture medium with naphthalene concentrations of (50, 100, and 150) pm was (6.0, 6.8, and 7.5) cm, respectively. The diameter of the control colony on the fifth day was (8.5) cm. The results indicated that neither fungus was influenced by the presence of naphthalene, which indicates the ability of the fungi to resist aromatic compounds and the results of the current study were similar to the results of Giraud et al., (2001), where he stated that aromatic compounds were not severely affecting the growth of the selected fungi in solid media, and also agreed with the results obtained by (Al-Jawhari, 2016; Al-Husseini, & Alsalman, 2020; Abdullah, & Kttafah, 2020) in which he showed the adaptation of fungi to solid media contaminated with the aromatic compound anthracene, and their ability to grow well.

Table 3: Facilitating increase in *A. flavus* and *P. fumigates* in solid medium

Fungi	Concentration (ppm)				Mean
	Control	50	100	150	
A. flavus	8.5±0.0	8.3±0.00	7.5±0.11	6.3±0.00	7.37
P. fumigates	8.5±0.0	7.5±1.0	6.8±0.28	6.0±0.0	6.76

Note: $P<0.05$ (L.S.D); L.S.D concentration = 0.0230; L.S.D fungi = 0.02

The Capacity of Secluded Fungus to Proliferate in Mineral Salts Media Augmented with Naphthalene

The results of the present study demonstrated that the isolated fungi exhibited good growth in the liquid mineral salts medium (SMS) supplemented with naphthalene. This indicates that these fungi are capable of growing in a liquid medium containing naphthalene at varying concentrations, although their growth rates differed among species. Statistical analysis confirmed that there were no significant differences in growth between fungal species or across the different naphthalene concentrations (Tables 4, 5).

It was observed that increasing naphthalene concentrations corresponded with an increase in fungal biomass. For example, the dry weight of *Aspergillus flavus* reached (2.05, 2.12, 2.25) g at concentrations of 50 ppm, 100 ppm, and 150 ppm, correspondingly, in relation to the control, which recorded a dry weight of (1.50 g). In contrast, *Penicillium fumigatus* showed a smaller increase in dry weight, reaching 1.95 g, 2.10 g, and 2.17 g at the same concentrations, with the control showing a dry weight of (1.30 g).

The study also found that the pH of the liquid media infused with *A. flavus* reached (6.5), while the medium inoculated with *P. fumigatus* reached a pH of 6.3. These findings are consistent with the results reported by Al-Jawhari (2015). It is well known that many fungi can grow over a wide pH range and that microbial degradation processes often produce organic acids and other metabolic by-products (Nwachukwu & Ugoji, 1995; Alhusainy & Kttafah, 2025).

Table 4: Capacity for development of *A. flavus* & *P. fumigates* in mineral salts medium

Fungi	Concentration (ppm)				Mean
	Control	50	150	250	
A. flavus	2.05±0.00	2.12±0.02	2.25±0.01	2.17±0.02	1.50
P. fumigates	1.95±0.00	2.10 ±0.01	2.17±0.02	2.05±0.05	8.10

Note: P<0.05 (L.S.D); L.S.D concentration = 0.00; L.S.D fungi = 0.100

Biodegradation of Naphthalene

The results of the current investigation revealed the capacity of fungus extracted from sediments and wastewater in the investigated region. to degrade the polycyclic aromatic hydrocarbon (PAH) naphthalene at concentrations of 50, 100, and 150 ppm in mineral salts medium.

Figures (2–7) show the biodegradation of naphthalene after seven days of incubation with *Aspergillus flavus* and *Penicillium fumigatus*. Compared to the standard naphthalene spectrum (Figure 1) significant changes were observed. Many characteristic peaks disappeared in the region (500–1500), and additional peaks vanished in the region (2500–3000), while two new peaks emerged. Additionally, that showed a broad band in the range (3000–3500) denotes the formation of hydroxyl (OH) groups, suggesting the production of organic acids. This may be associated with the fermentation of glucose in the culture media. These observations align with the findings of Al-Jawhari (2016) and Kanaly & Harayama (2000).

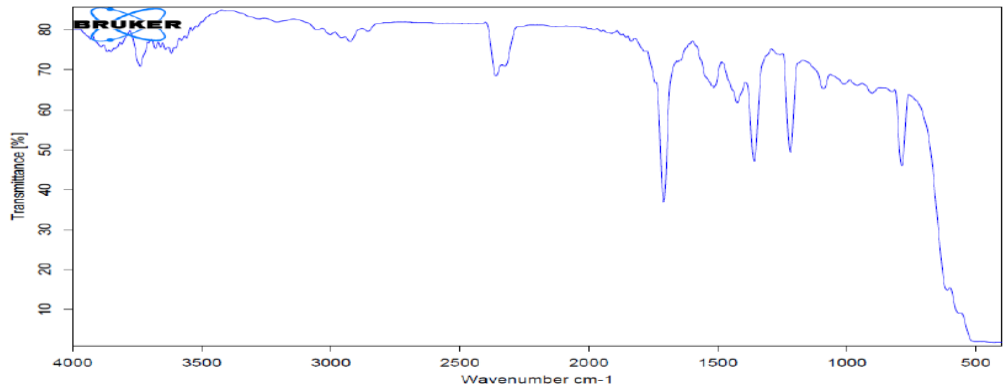

Figure 1: Naphthalene (standard)

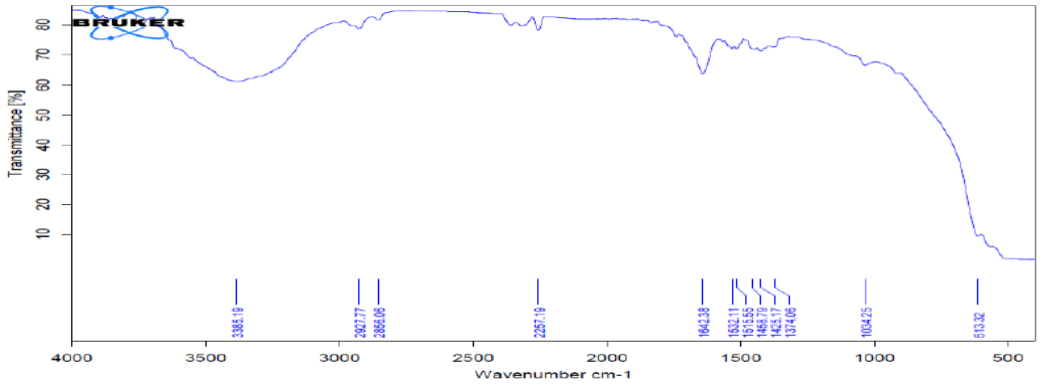

Figure 2: Biodegradation of Naphthalene by *A. flavus* after seven days incubation by (FTIR) at (50) ppm

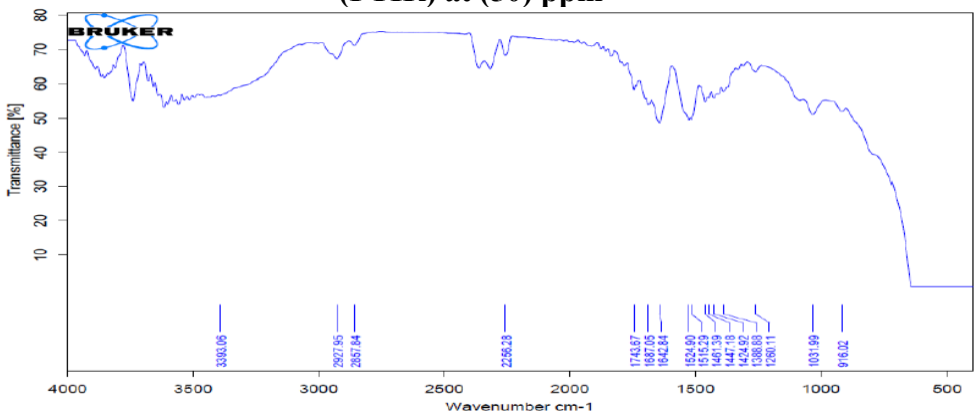

Figure 3: Biodegradation of Naphthalene by *A. flavus* after seven days incubation by (FTIR) at (100) ppm

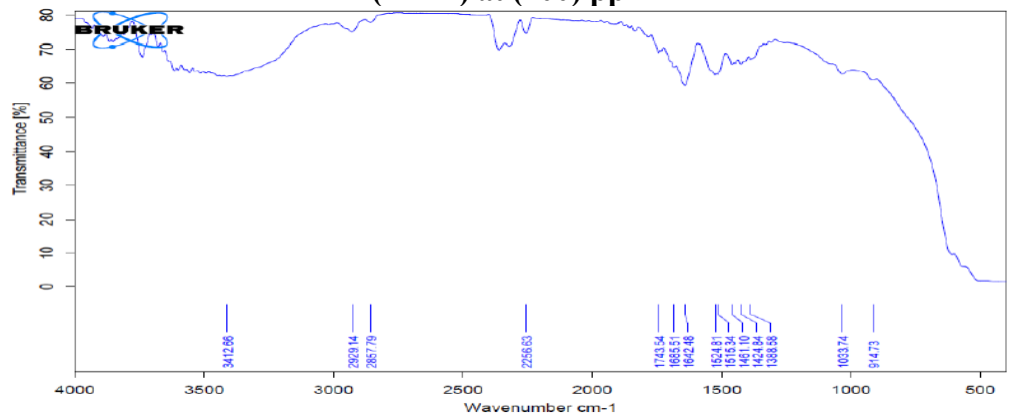

Figure 4: Biodegradation of Naphthalene by *A. flavus* after 7 days incubation by (FTIR) at (150) ppm

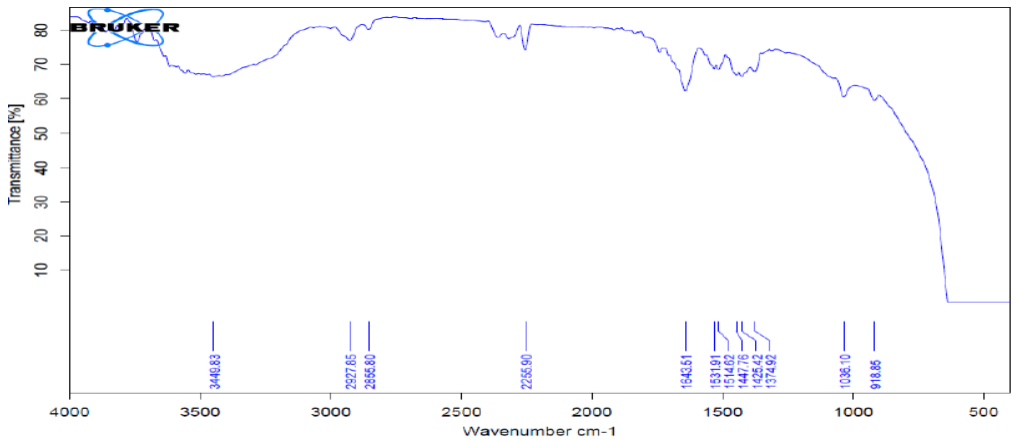

Figure 5: Biodegradation of Naphthalene by *P. fumigates* after seven days incubation by (FTIR) at (50) ppm

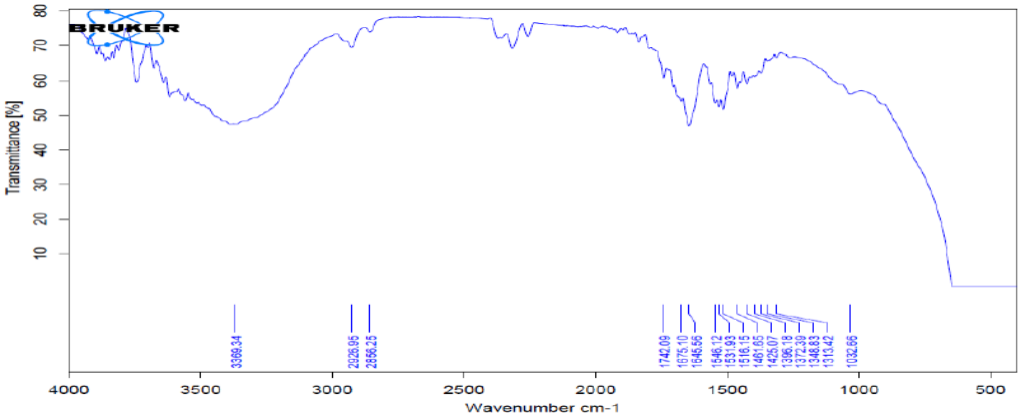

Figure 6: Biodegradation of Naphthalene by *P. fumigates* after seven days incubation by (FTIR) at (100) ppm

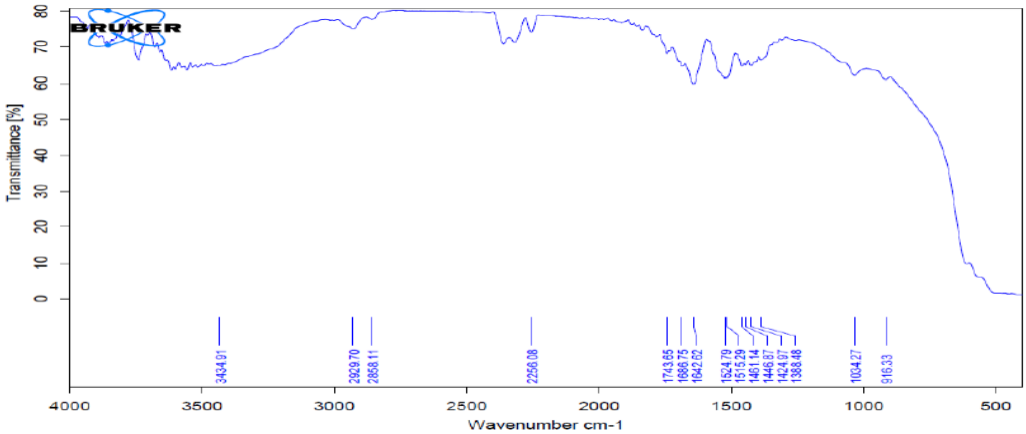

Figure 7: Biodegradation of Naphthalene by *P. fumigates* after seven days incubation by (FTIR) at (150) ppm

The results obtained from (GC-MS) analysis confirmed the ability of fungi isolated from wastewater and sediment samples from the Nasiriyah oil refinery and the Nasiriyah oil field (Al-Kati'a) to degrade the aromatic compound naphthalene. This degradation was observed at concentrations of 50, 100, and 150 ppm in mineral salts medium after a 7-day incubation period at 25°C. Figure (14) represents the standard (control) curve which represents the time of appearance of the compound in the technique GC-MS.

Figures (8, 9, 10) showed that *A. flavus* was able to analyze the compound Naphthalene. *A. flavus* was able to degrade Naphthalene at a concentration of (50,100,150) where the residual concentration was as shown in Table (5) (0.005,0.025,0.007) ppm with an analysis rate of (99.98, 99.70, 99.96) %, respectively, which indicates that the fungus was able to analyze the compound at all the mentioned concentrations.

Figures (11, 12, 13) showed the results of the degradation of Naphthalene compound by *P. fumigates* at concentrations of 50, 100 and 150ppm as the residual concentration as shown in Table (5) was as follows (0.003,0.020,0.005) ppm with an analysis rate of (99.99, 99.75, 99.97) % respectively where many metabolites were formed which differed in the retention time and this indicates that the fungus has the ability to analyze the compound at all the mentioned concentrations.

Table 5: Residual concentration of aromatic compound Naphthalene degradation by isolated fungi

Fungi	Residual concentration		
	50	100	150
A. flavus	0.005	0.025	0.007
P. fumigates	0.003	0.020	0.005

Note: L.S.D = 0.00

In this research, the examined fungus isolates demonstrated their capacity to decompose and eliminate the aromatic molecule naphthalene at varying rates within just seven days. These findings are consistent with previous research showing that many fungi belonging to the Ascomycota phylum such as *penicillium*, *Aspergillus*, *Pseudallescheria*, and *Fusarium* are frequently present in polluted environments however, possess the capability to break down pollutants in soils affected by industrial spills and fuel storage sites (Zafra et al., 2014; Azemi et al., 2021). The present results also align with the findings of Wick et al. (2011) & Al-Jawhari (2015).

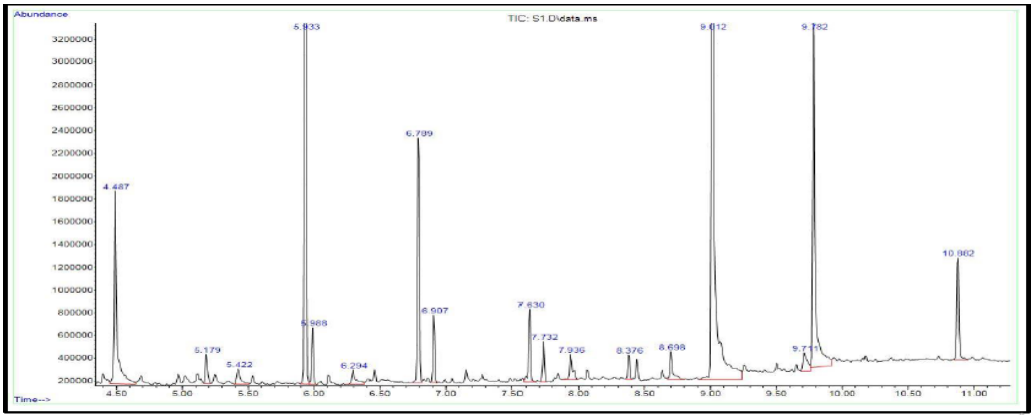

Figure 8: *A. flavus* GC-MS chromatogram following a 7- day incubation period with naphthalene treatment at a (50) ppm

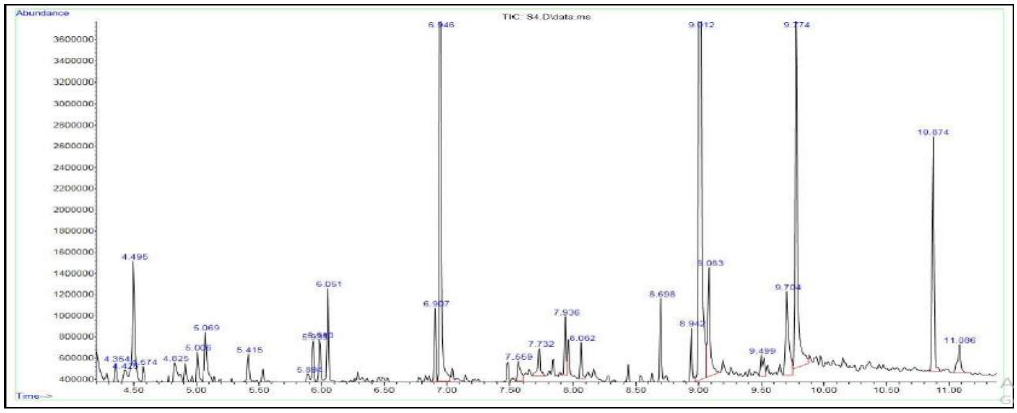

Figure 9: *A. flavus* GC-MS chromatogram following a 7- day incubation period with naphthalene treatment at a (100) ppm

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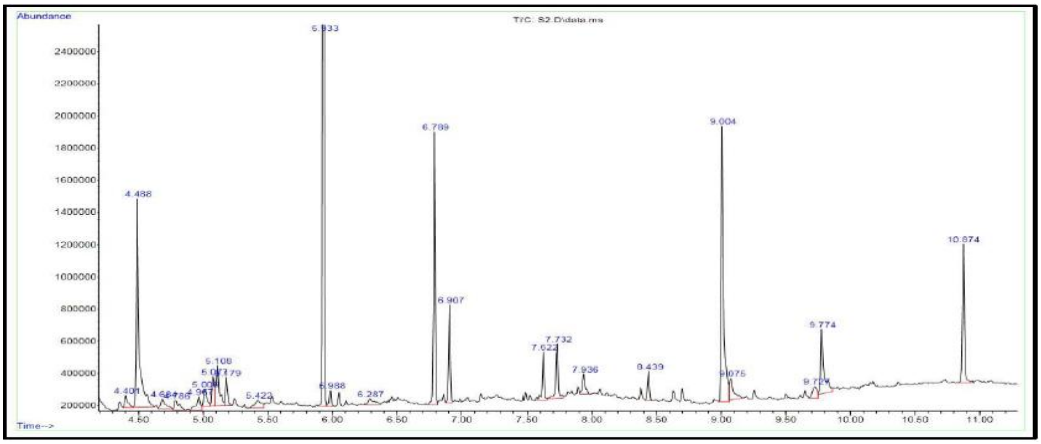

Figure 10: *A. flavus* GC-MS chromatogram following a 7- day incubation period with naphthalene treatment at a (150) ppm

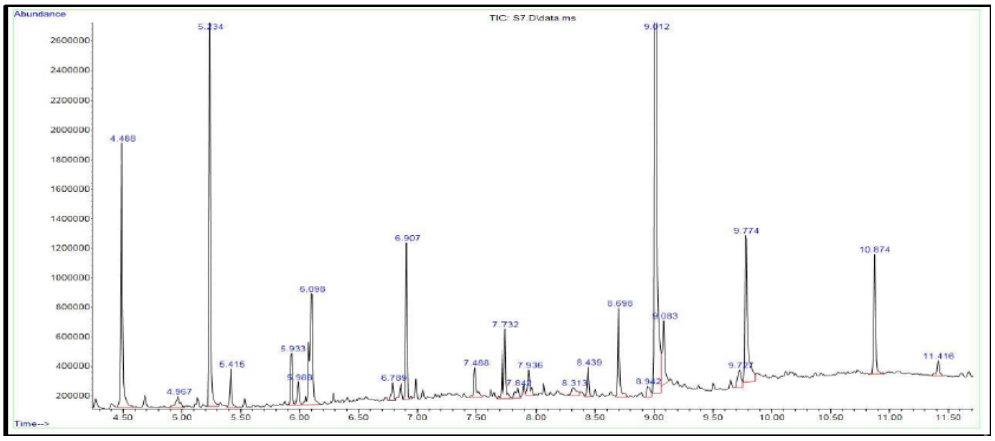

Figure 11: *P. fumigates* GC-MS chromatogram following a 7- day incubation period with naphthalene treatment at a (50) ppm

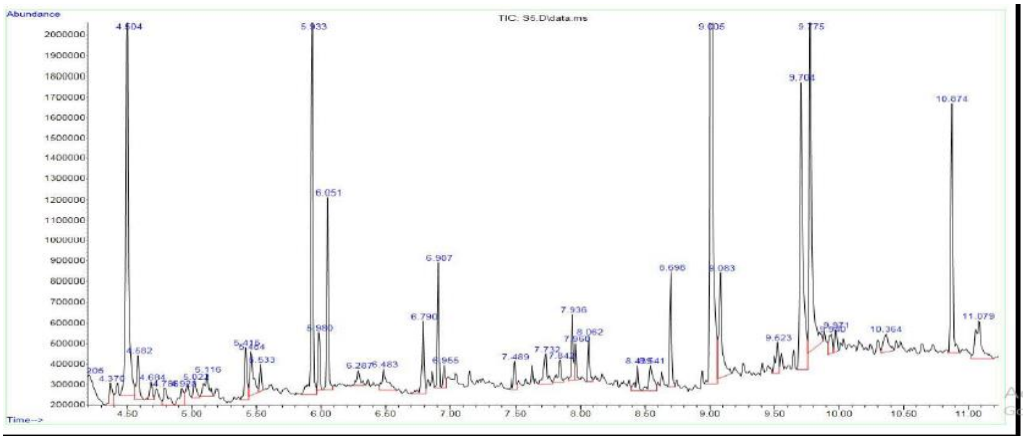

Figure 12: *P. fumigates* GC-MS chromatogram following a 7- day incubation period with naphthalene treatment at a 100 ppm

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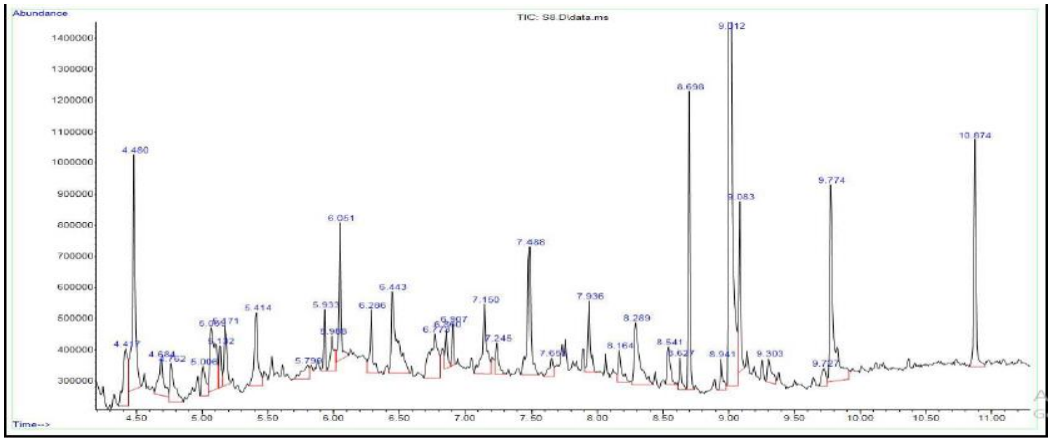

Figure 13: *P. fumigates* GC-MS chromatogram following a 7- day incubation period with naphthalene treatment at a (150) ppm

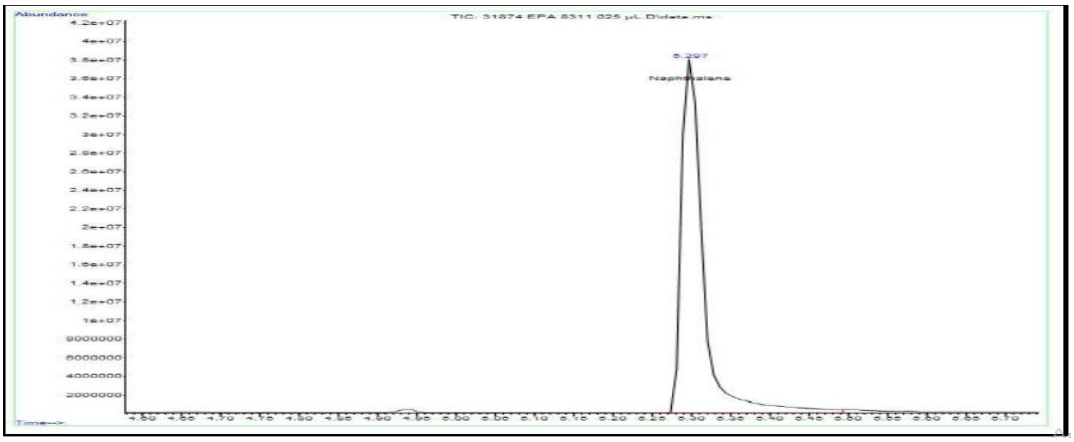

Figure 14: Gc-MS chromatography showed standard Naphthalene (control)

CONCLUSION

The biodegradation of chrysene by *Aspergillus flavus* and *Penicillium fumigatus* was assessed after seven days of Incubation in a mineral salt medium. The concentration of chrysene diminished to 0.03 ppm in the *A. flavus* culture, achieving a removal efficiency of 99.95%. In comparison, the chrysene concentration in the *P. fumigatus* culture dropped to 0.01 ppm, with a removal efficiency of 99.98%. The variation between sampling stations in this study had minimal impact on fungal diversity in the sediments, likely due to similar environmental conditions across all locations, including temp., DO, pH, and organic carbon levels, which were quantified at each site to assess their influence on fungal distribution. The findings from this study contribute valuable insights into the biodegradation of polycyclic aromatic hydrocarbons (PAHs) and enhance our understanding of fungal behavior in polluted marsh environments across different sites. These results also highlight the potential application of these fungal species for environmental bioremediation, both in current and future pollution management efforts.

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CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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