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Research article

Efficacy of *Lentinus tigrinus* for Kerosene Bioremediation

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Abstract

The strategy for removing pollutants in a sustainable and environmentally friendly manner, and the danger of the leakage of hydrocarbon compounds such as kerosene into the environment is important. Therefore, this study was focused on the potential of *Lentinus tigrinus* fungal biomass for the bioremediation of kerosene. A sample of mushroom *Lentinus tigrinus* was identified using morphlogical and molecular analyses. Kerosene was obtained from a petrol station (Al- thakafa) in the city of Mosul. *Lentinus tigrinus* fungal biomass was screened for its capability to degrade kerosene by culturing it on a solid medium supplied with kerosene at concentrations of 2, 4, and 6% compared to the control sample, and a liquid medium mineral salt medium (MSM). GC-MS chromatography was used to analyse the presence of kerosene before and after biodegradation process. After 7 days of incubation, *L. tigrinus* grew significantly at the probability level 0.01 on the medium supplemented with 2% kerosene (11.6 mm in diameter) compared with the control treatment without kerosense (14.83 mm in diamter). The result showed that kerosene was biodegraded into 15 compounds, most of which are hydrocarbon compounds, but in varying percentages depending on the retention time and percentage concentration of the compounds. The five most common compounds are (1) 1-heptanol, 2-propyl with a percentage of area of 8.60% , (2) 1-octanol 9.83%, (3) hexyl octadecyl ester 12.84%, (4) 7-methyl-1-undecene 12.05% and (5) isopropyl-5-methyl-1-hexanol 10.01%. While after treatment with the biomass of the fungal isolate after 14 days of incubation, kerosene was biodegraded into another 15 compounds with retention times and concentrations different from untreated kerosene. The most common of these compounds are (1) methyl 7,9-tridecadienyl ether 31.03%, (2) 4-fluoro-1-methyl-5-carboxylic acid 16.84%, and (3) triethylene glycol monododecyl ether 11.31%. From the results we can conclude that the biomass of the fungal isolate (identified morphologically and molecularly) has the ability to biologically degrade kerosene, whether on solid or liquid media.

Keywords: *Lentinus tigrinus*; kerosene; bioremediation; ITS; GC-MS chromatography

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1. Introduction

Environmental pollution that is associated with the use of petroleum hydrocarbons and their products (derivatives) is a controversial problem (Lednev et al.,2021). One of the most important derivatives is kerosene, which is widely used on a daily basis in cooking and lighting. It is also used as a solvent for grease and paint, as an aircraft fuel, and in the manufacture of pesticides. Kerosene is derived from petroleum oil by fractional distillation at 150-275°C. Despite its importance in daily life, it may be leaked into the environment through natural or anthropological processes (Bekele et al., 2022). This has a negative impact on the ecosystem and shows severe toxicity to living organisms as a result of transmission in the food chain, as well as its mutagenic and carcinogenic effect and their complex chemical and physical properties that make them difficult to decompose (Zhang et al., 2020). Moreover, one study confirmed that inhaling fumes emitted by kerosene and some other hydrocarbon compounds led to a clear change in lung tissue and negatively affected parts of the respiratory system (AL-Hakkak et al., 2024). The effect of kerosene on human health, especially on the central nervous system and some enzymes secreted by the liver, may be long-term or short-term and its carcinogenic effect must also be considered (Al-Hulfi et al., 2022). It is worth noting that soil pollution with kerosene significantly affects soil fertility and this is reflected in agricultural productivity (Kuzina et al., 2021). Petroleum hydrocarbons are considered a complex mixture of organic compounds of different molecular weights that affect soil properties and can have permanent effects on plants and wildlife or on the ecosystem in general (Brejea et al., 2023). Specialists have begun to pay serious attention to the severe imbalance that the environment is exposed to as a result of the continuous use of oil and its derivatives, especially aromatic ones (Thouand et al., 1999). The term bioremediation has been at the forefront of proposed strategies because it is an ideal, promising, environmentally friendly, low-cost, safe and successful strategy for removing toxic waste from the polluted environment without secondary pollution, and involving the use of biological agents (Bôto et al., 2021). Fungi play a leading role in the decomposition of hydrocarbon compounds because they possess extracellular enzymes that digest these complex compounds as substrates to obtain carbon as a source of energy necessary for metabolism, and this is called fungal bioremediation (mycoremediation) (Tomer et al., 2021). *Lentinus tigrinus* is one of the most important white rot mushrooms, which is known as tiger sawgill, belongs to Basidiomycota phylum, and Polyporales order. In general, it is a wild edible, wood-decaying, gilled mushroom distributed in tropical regions (Ragasa et al., 2018; Phonemany et al., 2021). There are about 63 species recognized all over the world (Ishaq et al., 2022), and of these species, *Lentinus tigrinus* mushroom is reported as the most widely distributed species that has been recorded since seventeen countries (Fabros et al., 2022). Moreover, Asif et al. (2024) confirmed that *Lentinus tigrinus* is one of the six species newly recorded in Pakistan. White-rot fungi have the ability to produce a collection of extracellular enzymes that degrade lignin such as lignin peroxidases, laccases and cellulase. That degradation plays an important role in breaking down lignin, a tough and difficult -to-degrade heteropolymer, which is found in plant cell walls. This is done in order to access the cellulose and hemicellulose, which re found in the lignin layer structure (Bautista-Zamudio et al., 2023).

2. Materials and Methods

2.1 Specimens collection

Lentinus tigrinus fruiting bodies were obtained from Mosul university gardens (Mosul- Iraq) during February-April, 2024. The fruiting bodies were found at a felled olive tree on the base of its trunk, in groups. The specimens were collected and placed in sterile zipper bags and then transported to the laboratory. Then, the fruiting bodies were washed carefully with tap water to remove all mud and dust stuck to them. The laboratory hood cabinet was sterilized with 70% ethanol. To obtain pure fungal colonies, a small piece from the heart of the fruiting body (hymenium layer) was taken and sterilized with 70% ethanol, inoculated on Potato Dextrose Agar (PDA) culture medium, then incubated at 28±2ºC for 5-10 days until the fungal colonies appeared (Kała et al., 2020).

2.2 Morphological identification

After taking photographs of the sample in the field, all the morphological characteristics of the fruiting body, size, color, gill shape, cap shape, texture and stem height, were recorded (Jarjees et al., 2023). The microscopic features including basidium, spore number and spore shape, were recorded. Lactophenol cotton blue dye was used for the compound light microscope. The fruiting body of the specimen was morphologically and microscopically identified (Watanabe, 2002; Phillips, 2013; Senthilarasu, 2015; Suliaman et al., 2017).

2.3 Molecular identification

To identity the fungal isolate by molecular methods, fungal mycelia from a newly growing fungal culture (7 days) was used. The genomic DNA was extracted using the extraction kit (Zymo Research, USA). The ITS region was amplified using the primers: forward ITS (5´-TCCGTAGGTGAACCTGCGG-3´) and reverse ITS4 (5´ - TCCTCCGCTTATTGATATGC-3´) (Hasan & Abdulhadi, 2022). The PCR product (655 bp) was checked by gel electrophoresis technique after preparing 1% agarose gel and then visualized by UV transilluminator in a dark room. The PCR product was sent to Macrogen Company, located in South Korea. When ITS sequence of *Lentinus tigrinus* was obtained, it was compared with the reference sequences in the GenBank database at the National Center for Biotechnology Information (NCBI). Our sequence was aligned with those nucleotide sequences from GenBank. The phylogenetic tree was constructed by the program MEGA X software. Evolutionary history was inferred using the Minimum Evolution method (Rzhetsky & Nei 1992). The optimal tree is shown. The confidence probability (multiplied by 100) that the interior branch length is greater than 0, as estimated using the bootstrap test (500 replicates is shown next to the branches) (Rzhetsky & Nei 1992; Dopazo,1994).

2.4 Culture media

2.4.1 Liquid media: Mineral salt medium (MSM)

MSM medium was prepared by dissolving the following ingredients in 1,000 mL of distilled water (g/L): 10 NaCl, 0.42 $MgSO_4$, 0.12KCl, 0.83KH_2PO_4, 0.42 $NaNO_3$, 1.25 Na_2HPO_4, with 0.1% (v/v) of Tween 80 as a stimulant, and 0.6 mg/mL of the redox indicator 2,6-

dichlorophenolindophenol (DCPIP) (Al-Dossary et al., 2019; Al-Otibi et al., 2022). This medium is considered specific for the analysis of hydrocarbon compounds.

2.5 Bioremediation of kerosene

A modified DCPIP assay was used to evaluate the ability of the fungal isolate to degrade kerosene. Kerosene was obtained from a petrol station (Al- thakafa) in the city of Mosul, transported to the laboratory and stored in dark opaque glass bottles at laboratory temperature until used. The steps for bioremediation of kerosene were carried out as follows.

2.5.1 Solid media

PDA culture medium was aliquoted into 250 mL glass flasks at 100 mL PDA/flask. After sterilization, PDA medium was added with kerosene at concentrations of 2, 4, or 6%. Kerosene was prior sterilized by filtering through a membrane filter with a diameter of 0.45 µm. The filtration was done inside the culture cabinet. One of the flasks was left without adding kerosene (negative control). Each mixture was distributed in sterile petri dishes in three replicates for each concentration. Then, the dishes were inoculated with a disc of newly grown fungus colony with a diameter of 6 mm. The dishes were incubated at a temperature of 28±2°C for 7 days. After the end of the incubation period, the diameter of the colony growth was measured and compared with the control treatment (Hasan, 2014).

2.5.2 Liquid media

Mineral salt medium (MSM) was aliquoted into 250 mL conical flasks (100 mL/flask). After sterilizing the medium, kerosene concentrations of 2, 4 and 6 % sterilized with a membrane filter with a diameter of 0.45 µm, were added separately in each flask. Also 0.6 mg/mL of the redox indicator 2,6-dichlorophenolindophenol (DCPIP) was added (Al-Otibi et al., 2022). All treatments were performed in triplicate. Each flask was inoculated with 6 agar plugs of a newly grown fungus colony using a cork borer with a diameter of 6 mm. One flask was left uninoculated as the control treatment. The flasks were incubated in a shaking incubator at 28±2°C with shaking speed at 150 rpm for 14 days. At the end of the incubation period, the contents of the flasks were filtered using a vacuum device after attaching filter papers to a Buchner funnel. To calculate biodegradation efficiency %, a portion of the filtrate was taken and its absorbance was measured at a wavelength of 420 nm, which was observed by the color of DCPIP changing from blue to colorless according to the method of Oudot (1984) as in the following equation.

$$\text{Biodegradation efficiency (\%)} = (\text{amount of kerosene in period zero} - \text{amount of kerosene after 14 days/amount of kerosene in period zero}) \times 100$$

To separate the kerosene from the liquid medium, the filtrate was poured into a separating funnel and the organic solvent petroleum ether and acetone were added to it in a ratio of 1:1, shaking well for 10 min carefully until two layers formed. During that process the valve was opened several times to expel gases. The funnel was left for 5 min for the solution to settle. The lower layer, which contained water, was neglected. As for the upper layer, which contained kerosene and the solvent, it was subjected to a purification process using a separation column equipped with glass wool topped with a layer of alumina, to get rid of fatty acids, then a layer of anhydrous sodium sulphate to absorb water from the

sample. Then, the sample was left to dry (Mittal & Singh, 2009). The dried sample was dissolved in ethyl acetate and analyzed using gas chromatography-mass spectrometry (GC-MS) (Kaczorek et al., 2014).

2.6 Gas chromatography-mass spectrometry (GC-MS) analysis

The extracted sample was analyzed for the presence of kerosene before and after biodegradation process using gas chromatography-mass spectrometry (GC-MS) by injecting 1 µL of the extract. A GC-MS – QP2010 Plus device which was equipped with an FID flame detector with a flow rate of 1.69 mL/min, was used. The temperature was set to 210°C, and helium was used as the directing gas, at a pressure 100 kPa, and a split ratio 10:1. The capillary separation column was made of quartz with dimensions of 30 m (length) x 0.25 mm (ID) x 0.25 µm (thickness). The compounds were identified by comparing them to the control treatment based on the retention index for each compound. The electron bombardment energy was 72 volt, with charging at 500 volt. The m / z scan range was from 40 to 650 at 3 scans/s and the data were evaluated using total ion counts (TIC) for compound identification and spectra quantification. Compounds were identified by comparing them to substances of known structure in the spectrum database stored in the device's library and by looking at the retention index for each compound (Kaczorek et al., 2014).

3. Results and Discussion

3.1 Phenotypic microscopic ıdentification

Lentinus tigrinus is also known as tiger sawgill or kabuteng tigre due to the brown scales on the surface of its cap (Dulay & De Castro, 2016). *Lentinus tigrinus* is an edible mushroom belonging to the family Polyporaceae. It is the most widespread rotting basidiomycetes (Liwanag et al., 2020). The caps were 2.5-4.5 cm in diameter, 1.5-2 cm in thickness, and round in shape. They had curved smooth edges, and their color of the cap ranged from white to cream in the center. There were light brown scales, which looked like teeth, scattered on the cap. Mushroom gills were attached to the stipe, and were of crowded gill spacing (Figure 1). The stipe was cylindrical shape, 1.5-3.5 cm in height, and 1.2- 3.5 mm in diameter. Each stioe was fibrous in texture, white or cream in color, and several stipes arising from a felled trunk. Basidiospores usually are four oval spores held by the cylindrical basidia with the dimensions of 12- 17 × 3.5-4.5 µm (Dulay et al., 2020; Fabros et al., 2022).

3.2 Molecular identification

After completing the stages of electrophoresis process on agarose gel, distinctive bright horizontal bands appeared under ultraviolet light (UV) due to the strong attachment of the bright ethidium bromide dye to the DNA. The results shown in Figure 2 confirmed that the band molecular weight was 655 bp compared to the ladder which clearly proved that the ITS region was amplified successfully by Polymerase Chain Reaction PCR technique (Abdulhadi et al., 2020). The ITS nucleotide sequences of *L. tigrinus* received from "Macrogen Company" in high quality was listed in GenBank website (http://www.ncbi.nlm.nih.gov/blast), with accession number OQ099798 that showed a match of 100% with nucleotide sequences deposited in GenBank (the accession: OW988497.1) (Figure 3). The evolutionary tree was inferred by using the Minimum

Evolution method (Rzhetsky & Nei 1992). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004) and were in the units of the number of base substitutions per site. The ME tree was searched using the Close-Neighbor-Interchange (CNI) algorithm (Nei & Kumar, 2000) at a search level of 1. The Neighbor-joining algorithm (Saitou & Nei, 1987) was used to generate the initial tree. The proportion of sites where at least 1 unambiguous base is present in at least 1 sequence for each descendent clade is shown next to each internal node in the tree. This analysis involved 20 nucleotide sequences. Codon positions included were 1st+2nd+3rd+ Noncoding. All positions containing gaps and missing data were eliminated (complete deletion option). There was a total of 429 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar et al., 2018).

Figure 1. *Lentinus tigrinus* (a) Mature fruiting bodies; (b) Fungal colony on culture medium; (c) Mushroom crowded gills; (d) Clamp connection on hypha; (e) The basidium and Basidiospores. Scale bars: (a,c) 1 cm , (e) 12- 17 ×3.5-4.5 µm

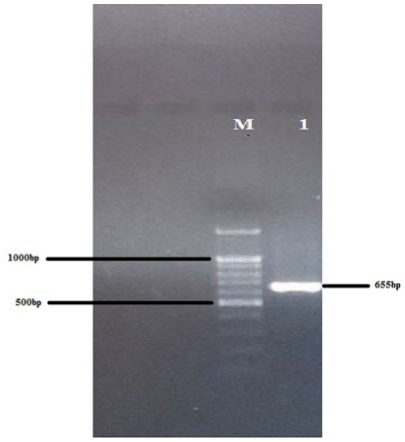

Figure 2. Molecular weight of amplified DNA (655 bp) of *Lentinus tigrinus* fungus on 1% agarose gel as bright bands. M: the ladder, 1: DNA band

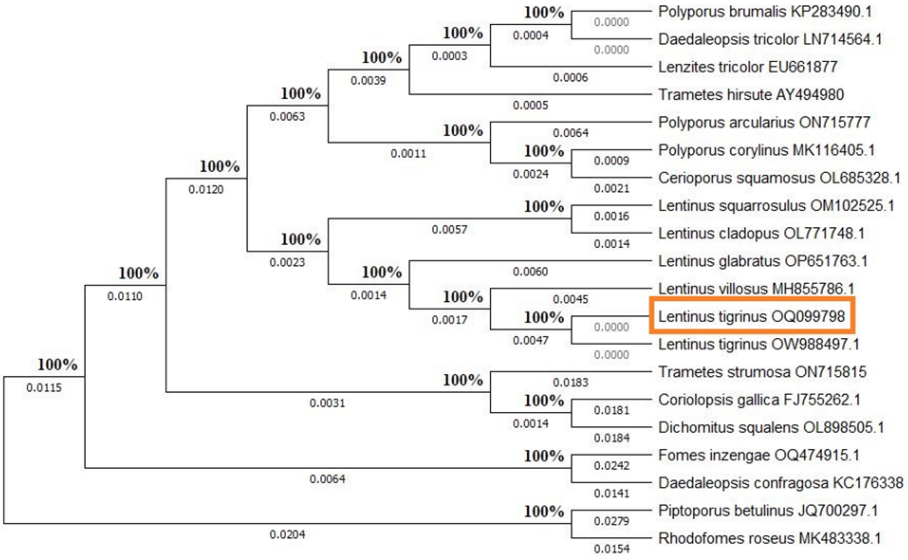

Figure 3. Minimum likelihood phylogenetic tree of *Lentinus tigrinus* (OQ099798) (indicated in red rectangle) with some deposited sequences in NCBI based on ITS sequences

3.3 Bioremediation of kerosene

3.3.1 Solid and liquid media

The results in Table 1 show that the diameters of fungal colonies were inversely proportional to the increase in kerosene concentrations (2, 4, 6%) after 7 days of incubation. The diameter of the fungal colony reached 11.6 mm at a concentration of 2% kerosene, showing a high level of significant growth at the probability level of 0.01 compared to the control sample which reached 14.83 mm and the other concentrations (4, 6%) with diameters of fungal colonies of 10.32 mm and 9.53 mm, respectively. The degradation of kerosene by the biomass of the isolate *L. tigrinus* was determined in the liquid medium after 14 days of incubation at 28±2°C and speed shaking at 150 rpm by the isolate at the concentration 2% which gave the best percentage of biodegradation (Table 2).

The results in Table 2 show that the highest percentage of biodegradation was at a concentration of 2% kerosene, which was 69.55% compared to the other concentrations which reached 44.36% and 27.11% for the concentrations of 4% and 6% kerosene, respectively. The densely growing fungal hyphae may have been able to attack the complex compounds of kerosene at the studied concentrations and decompose them into simple compounds and use the high carbon content of kerosene as the only source of energy by secreting extracellular enzymes as one of the metabolic mechanisms and producing easily absorbed substances with less toxic effect on the ecosystem. This is consistent with Hasan (2014) in the ability of two fungal isolates *Rhizopus stolonifer* and *Aspergillus niger* growth on potato dextrose agar (PDA) medium containing 0, 5%, 10%, 15%, and 20% (v/v) of kerosene to biodegrade kerosene. The highest diameter of fungal colony growth of the fungus *R. stolonifer* reached 8.5 cm after 7 days incubation.

Table 1. Colonies diameters of *Lentinus tigrinus* fungus grown on solid medium (PDA) supplied with kerosene concentrations of 2, 4, and 6%

Concentrations of Kerosene%	Average Diameter of Growing Colony* (mm) ± SD
Control	14.83±0.856[a]
2	11.6±1.828[b]
4	10.32±0.966[b]
6	9.53±0.625[b]

*Significance at probability level 0.01. a, b: All transactions with similar letters have no significant differences, while those with different letters have significant differences at the probability level 0.01.

Table 2. Percentage (%) of biodegradation of kerosene concentrations

Concentrations of Kerosene%	The Percentage of Biodegradation%
2	69.55
4	44.36
6	27.11

3.3.2 Gas chromatography-mass spectrometry (GC-MS)

After injecting the untreated kerosene sample (control) into the GC device and based on the number of peaks that appeared in the device diagram, it turned out that kerosene sample degraded to 15 chemical substances (Figure 4), and five main peaks were found. The mass spectra indicated the presence of five compounds that were considered to have the highest percentage of area and peak height, in addition to the concentrations of these compounds compared to other compounds. 1-Heptanol, 2-propyl in peak (1) had the percentage of area of 8.60%, height of 7.57% and retention time of 4.298 min, while 1-octanol in peak (3) had the percentage of area of 9.83%, height of 6.52% and retention time of 5.252 min. Hexyl octadecyl ester in peak (6) had the percentage of area of 12.84%, height of 9.20% and retention time of 5.878 min while 7-methyl-1-undecene in peak (10) had the percentage of area of 12.05%, height of 8.29% and retention time of 7.480 min. Finally, isopropyl-5-methyl-1-hexanol in peak (14) had the percentage of area of 10.01%, height of 8.61% and retention time of 9.025 min (Table 3 and Figure 5).

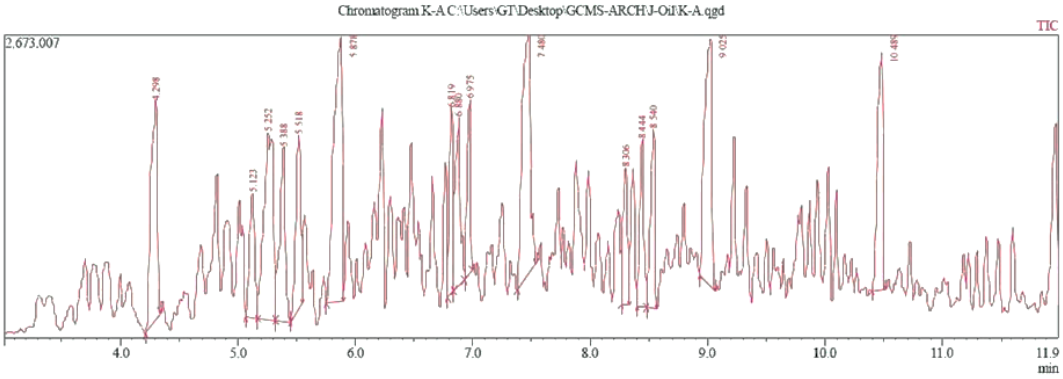

Figure 4. Peaks of chemical compounds which were separated from kerosene before treatment with the biomass of the fungus *L. tigrinus* using GC-MS technology

Table 3. Identification of chemical components of kerosene before treatment with the biomass of the fungus *L. tigrinus* using GC-MS technology

Peak	Retention Time	Area	Area%	Height %	Name
1	4.298	7797039	8.60	7.57	1-Heptanol, 2-propyl*
2	5.123	3437739	3.79	4.35	Hydroxylamine
3	5.252	8920175	9.83	6.52	1-Octanol*
4	5.388	5337012	5.88	6.05	Dodecyl isohexyl ester
5	5.518	4507060	4.97	6.01	Trichloroacetic acid
6	5.878	11646578	12.84	9.20	Hexyl octadecyl ester*
7	6.819	3498493	3.86	6.44	1-Fluorononane
8	6.880	4210001	4.64	5.87	3,7-Dimethyl
9	6.975	3504473	3.86	6.05	Decyl propyl ester
10	7.480	10926956	12.05	8.29	7-Methyl-1-undecene*
11	8.306	3656095	4.03	4.77	2-Tetradecyl ester
12	8.444	3478019	3.83	5.82	6-Methyl-2-heptanol, trifluoroacetate
13	8.540	3998288	4.41	6.23	Dichloroacetic acid
14	9.025	9083392	10.01	8.61	Isopropyl-5-methyl-1-hexanol*
15	10.489	6713688	7.40	8.22	Oxalic acid
		90715008	100.00		

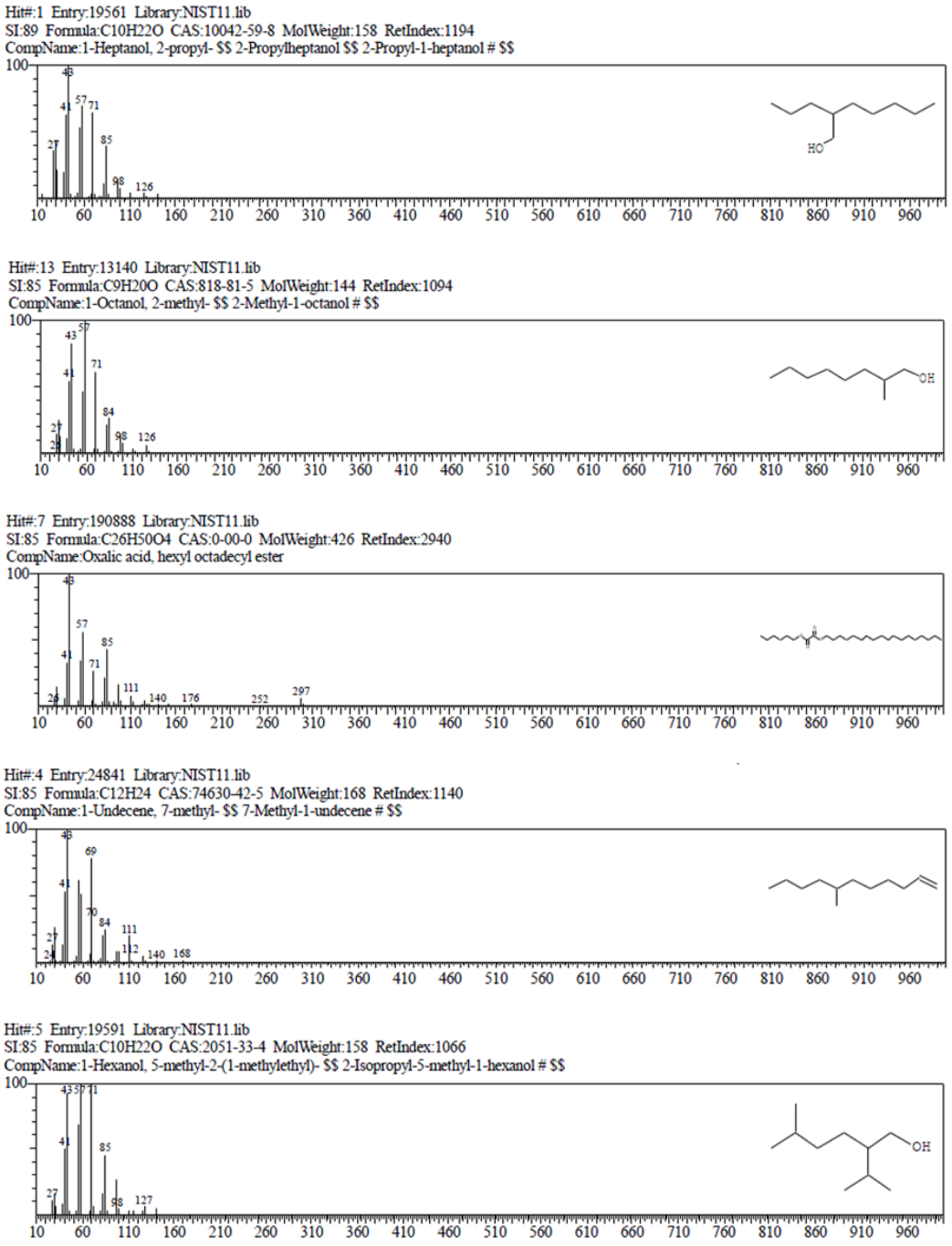

Figure 5. Mass spectrum of the chemical compounds that were the highest concentrations in the untreated kerosene sample (control)

The results listed in Table 4 confirmed that the kerosene sample treated with the biomass of the fungus *L. tigrinus* biodegraded into 15 chemical substances based on the number of peaks seen in Figure 6. In addition to that, the mass spectrometry results showed the identification of these compounds and included are their names, molecular weights, chemical compositions and the percentage of area they occupy. Three main peaks were found. These peaks represent the highest percentage of area, height and concentration compared to other compounds. Methyl 7,9-tridecadienyl ether in peak (10) had the highest percentage of area of 31.03%, height of 39.34% and retention time of 9.821 min. Next, 4-fluoro-1-methyl-5-carboxylic acid located in peak (12) had the percentage of area of 16.84%, height of 15.50% and retention time of 10.773 min. Last was triethylene glycol monododecyl ether in peak (5), which had the percentage of area of 11.31%, height of 9.68% and retention time of 8.252 min (Figure 7). The technique of GC-MS was used to detect the hydrocarbon compounds of kerosene because of the high accuracy and flexibility of this technique, as it combines gas chromatography with the mass spectrometry (Das & Chandran, 2011). When comparing the results shown in Figures 4 and 6, we noticed that the treatment with biomass of the fungal isolate reduced the area under each peak at different retention times**.** This may indicate the removal of hydrocarbon compounds with high numbers of carbon atoms as a result of them being metabolized by the fungus, as the highest and sharpest peaks correspond to alkanes, while the peaks located between them indicate aromatic compounds (Khan et al., 2015). The variation in number and type of compounds which showed the highest percentage of area for the kerosene sample before and after treatment with the *L. tigrinus* fungal biomass can be attributed to its special powerful and promising ability to remove toxic compounds that pollute the environment in sustainable ways by attacking the organic pollutants and converting them via degradation pathways within metabolism to meet their needs in the biosynthesis of living mass (Raju & Scalvenzi, 2017). In addition to that, the fungus has the ability to form a water**-**repellent fungal network that covers several hectares of soil which enhances its efficiency in reaching hydrocarbon pollutants. It also has the ability to degrade these compounds with its highly precise extracellular enzyme system and take pollutants as substrates and thus as the sole sources of carbon (Daccò et al*.,* 2020). In this regard, Mohammed et al*.* (2023) mentioned the ability of fungal isolates *Aspergillus lentulus* and *Rhizopus arrhizus* to biodegrade pure hydrocarbon compounds in contaminated soils by the 2,6**-**dichlorophenol indophenol (DCPIP) method and GC–MS technique.

Table 4. Identification of chemical components of kerosene after treatment with the biomass of the fungus *L. tigrinus* using GC-MS technology

Peak	Retention Time	Area	Area%	Height %	Name
1	3.640	51864	0.60	1.62	Ethyl dodecyl ether
2	4.988	127934	1.48	3.25	1,2,4-Triazole
3	7.615	391841	4.52	3.11	Oxirane
4	7.943	380755	4.39	4.30	Ethyl tetradecyl ether
5	8.252	980271	11.31	9.68	Triethylene glycol monododecyl ether*
6	8.564	527250	6.08	6.32	6,10,13-Trimethyltetradecyl isovalerate
7	9.088	167403	1.93	2.32	Octadecanoic acid
8	9.400	170365	1.97	1.22	Methyl 6,8-dodecadienyl ether
9	9.605	158299	1.83	1.93	3-Trifluoroacetoxypentadecane
10	9.821	2689012	31.03	39.34	Methyl 7,9-tridecadienyl ether*
11	10.138	117829	1.36	1.71	Dimethyl-1-decanol
12	10.773	1459138	16.84	15.50	4-Fluoro-1-methyl-5-carboxylic acid*
13	11.314	1151813	13.29	5.54	Decan-2-yl propyl carbonate
14	11.647	151415	1.75	1.62	2-Heptafluorobutyroxypentadecane
15	11.835	139935	1.61	2.56	Ethanol, 2-[2-(dodecyloxy)ethoxy]
		8665124	100.00		

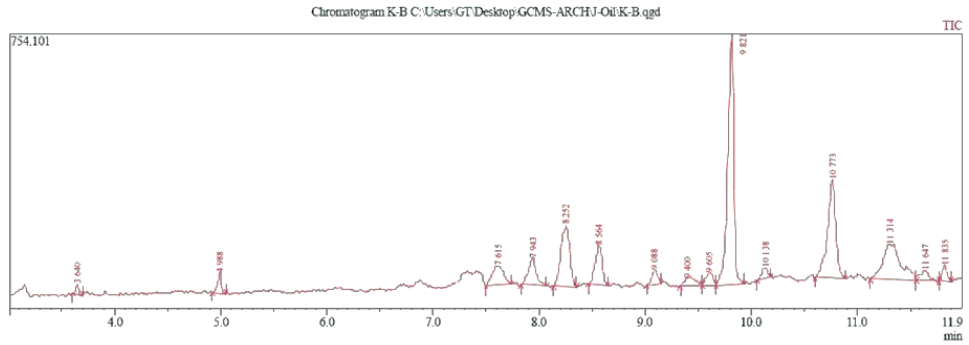

Figure 6. Peaks of chemical compounds which were separated from kerosene sample that was treated with the biomass of the fungus *L. tigrinus* using GC-MS technology.

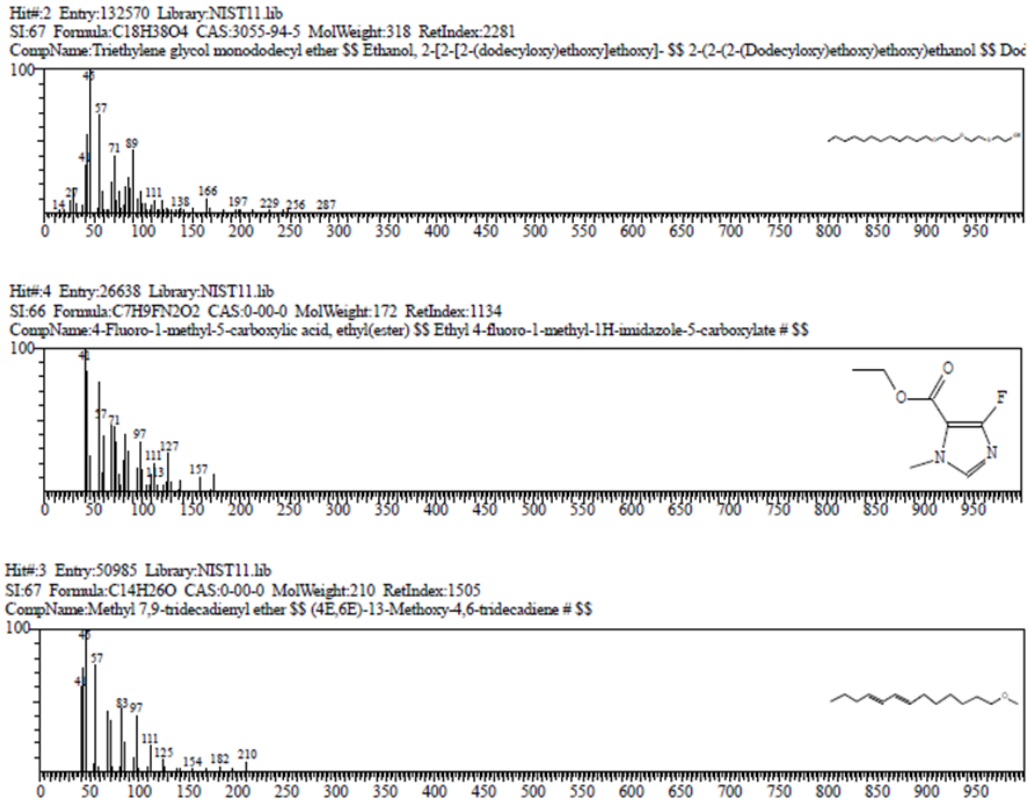

Figure 7. Mass spectra of the chemical compounds that were at the highest concentrations in kerosene sample treated with the biomass of the fungus *L. tigrinus*.

4. Conclusions

Lentinus tigrinus biomass demonstrated an effective ability to biodegrade kerosene at the studied concentrations of 2, 4, and 6% after 14 days of incubation. The diameter of the fungal colony on solid medium (PDA) reached 11.6 mm at a concentration of 2% kerosene, showing a significant growth compared to the control sample, which reached 14.83 mm. The highest percentage of biodegradation was at a concentration of 2% kerosene, which reached 69.55% compared to the other concentrations. On liquid medium, a variation was observed in the number and type of chemical compounds of kerosene before and after treatment with the biomass. The results of the GC-MS analysis showed that the biomass of the fungal isolate reduced the area under each peak at different retention times. This may indicate the removal of hydrocarbon compounds with high carbon atoms as a result of their metabolism by the fungus. This was probably due to the utilization of these compounds as the only carbon source consumed by the fungal isolate to carry out metabolic processes. The effective ability of biodegradation of *L. tigrinus* biomass proved that it can be used for kerosene mycoremediation.

5. Acknowledgements

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6. Conflicts of Interest

There are no conflicts of interest.

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References

Abdulhadi, S. Y, Gergees, R. N, & Hasan, G. Q. (2020). Molecular identification, antioxidant efficacy of phenolic compounds, and antimicrobial activity of beta-carotene isolated from fruiting bodies of *Suillus* sp. *Karbala International Journal of Modern Science*, 6(4), Article 4. https://doi.org/10.33640/2405-609X.1966

Al-Dossary, M. A., Abood, S. A., & AL-Saad, H. T. (2019). Biodegradation of crude oil using *Aspergillus* species. *Journal of Biology, Agriculture and Healthcare*, 9(4, 60-64. https://doi.org/10.7176/JBAH/9-4-09

AL-Hakkak, Z. M, Jasim, S. A., Kadum, A. A. (2024). The impact of kerosene and naphtha fumes inhalation on lung tissue in rats. *Biological and Biomedical Journal*, 2(2), 115-121.

Al-Hulfi, R. A., Al Salem, B. A. M., & Al-Naiema, I. M. (2022). The effect of air pollutants on liver enzymes and pituitary gland hormones of smokers and non-smokers of oil refinery and gas station workers in Basra/ Iraq. *Indian Journal of Forensic Medicine and Toxicology*, 16(3), 297-301. https://doi.org/10.37506/ijfmt.v16i3.18301

Al-Otibi, F., Al-Zahrani, R. M., & Marraiki, N. (2022). The crude oil biodegradation activity of *Candida* strains isolated from oil-reservoirs soils in Saudi Arabia. *Scientific Reports*, 12(1), Article 10708. https://doi.org/10.1038/s41598-022-14836-0

Asif, M., Saba, M., Maula, F. & Raza, M. (2024). *Lentinus punjabensis* (Polyporaceae), a new species from Lal Suhanra National Park, southern Punjab, Pakistan. *Phytotaxa*, 642(2), 169-181. https://doi.org/10.11646/phytotaxa.642.2.5

Bautista-Zamudio, P. A., Flórez-Restrepo, M. A., López-Legarda, X., Monroy-Giraldo, L. C., & Segura-Sánchez, F. (2023). Biodegradation of plastics by white rot fungi: A review. *Science of the Total Environment*, 901, Article 165950. https://doi.org/10.1016/j.scitotenv.2023.165950

Bekele, G. K., Gebrie, S. A., Abda, E. M., Sinshaw, G., Haregu, S., Negie, Z. W., Tafesse, M., & Assefa, F. (2023). Kerosene biodegradation by highly efficient indigenous bacteria isolated from hydrocarbon-contaminated sites. *Microbiology Insights*, 16, 1-9. https://doi.org/10.1177/11786361221150759

Bôto, M. L., Magalhães, C., Perdigão, R., Alexandrino, D. A. M., Fernandes, J. P., Bernabeu, A. M., Ramos, S., Carvalho, M. F., Semedo, M., La Roche, J., Almedia, C. M. R., & Mucha, A. P. (2021). Harnessing the potential of native microbial communities for bioremediation of oil spills in the Iberian Peninsula NW coast. *Frontiers in Microbiology*, 12, Article 633659. https://doi.org/10.3389/fmicb.2021.633659

Brejea, R., Boroș, M., Roșca, S., Traian, J. E., Budău, R., Borza, I. M., & Păcurar, I. (2023). Bioremediation of oil contaminated soil and restoration of land historically polluted with oil products in the agricultural circuit in the Plain and Western Hills, Romania. *Applied Sciences*, 13(18), Article 10245. https://doi.org/10.3390/app131810245

Daccò, C., Girometta, C., Asemoloye, M. D., Carpani, G., Picco, A. M., & Tosi, S. (2020). Key fungal degradation patterns, enzymes and their applications for the removal of aliphatic hydrocarbons in polluted soils: A review. *International Biodeterioration and Biodegradation*, 147, Article 104866. https://doi.org/10.1016/j.ibiod.2019.104866

Das, N., & Chandran, P. (2011). Microbial degradation of petroleum hydrocarbon contaminants: An overview. *Biotechnology Research International*, 2011, Article 941810.

Dopazo, J. (1994). Estimating errors and confidence intervals for branch lengths in phylogenetic trees by a bootstrap approach. *Journal of Molecular Evolution*, 38, 300-304.

Dulay, R. M. R., Cabrera, E. C., Kalaw, S. P., Reyes, R. G., & Hou, C. T. (2020). Nutritional requirements for mycelial growth of three *Lentinus* species from the Philippines. *Biocatalysis and Agricultural Biotechnology*, 23, Article 101506. https://doi.org/10.1016/j.bcab.2020.101506

Dulay, R. M. R., & De Castro, M. E. G. (2016). Effect of lead (Pb) in mycelia and sporocarp of *Lentinus tigrinus* mushroom. *International Journal of Biology, Pharmacy and Allied Sciences*, 5(6), 1323-1331.

Fabros, J. A., Dulay, R. M. R., De Leon, A. M., Kalaw, S. P., & Reyes, R. G. (2022). Distribution, cultivation, nutritional composition, and bioactivities of *Lentinus* (Polyporaceae, Basidiomycetes): A review. *Current Research in Environmental and Applied Mycology*, 12(1), 170-219. https://doi.org/10.5943/cream/12/1/13

Hasan, G. Q., & Abdulhadi, S. Y. (2022). Molecular characterization of wild *Pleurotus ostreatus* (MW457626) and evaluation of β-glucans polysaccharide activities. *Karbala International Journal of Modern Science*, 8(1), 52-62.

Hasan, I. F. (2014). Biodegradation of kerosene by *Aspergillus niger* and *Rhizopus stolinifer*. *Journal of Applied and Environmental Microbiology*, 2(1), 31-36. https://doi.org/10.33640/2405-609X.3204

Ishaq, M., Galappaththi, M. C. A., Khan, M. B., Ullah, S., Fiaz, M., & Khalid, A. N. (2022). *Lentinus squarrosulus* an edible macro-fungus reported from Pakistan. *Studies in Fungi*, 7(1), Article 6. https://doi.org/10.48130/SIF-2022-0006

Jarjees, R. M., Abdul-Hadi, S. Y., & Al-Khesraji, T. O. (2023). First recording and molecular diagnostics of three ascomycetous macrofungi from Nineveh, Iraq. *Journal of Advanced Education and Sciences*, 3(3), 36-44.

Kaczorek, E., Bielicka-Daszkiewicz, K., Héberger, K., Kemény, S., Olszanowski, A., & Voelkel, A. (2014). Best conditions for biodegradation of diesel oil by chemometric tools. *Brazilian Journal of Microbiology*, 45, 117-126.

Kała, K., Kryczyk-Poprawa, A., Rzewińska, A., & Muszyńska, B. (2020). Fruiting bodies of selected edible mushrooms as a potential source of lovastatin. *European Food Research and Technology*, 246, 713-722. https://doi.org/10.1007/s00217-020-03435-w

Khan, S. R., Nirmal, J. I. K., Kumar, R. N., & Patel, J. G. (2015). Biodegradation of kerosene: Study of growth optimization and metabolic fate of *P. janthinellum* SDX7. *Brazilian Journal of Microbiology*, 46(2), 397-406. https://doi.org/10.1590/S1517-838246220140112

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, 35(6), 1547-1549. https://doi.org/10.1093/molbev/msy096

Kuzina, E., Rafikova, G., Vysotskaya, L., Arkhipova, T., Bakaeva, M., Chetverikova, D., Kudoyarova, G., Korshunva, T., & Chetverikova, S. (2021). Influence of hydrocarbon-oxidizing bacteria on the growth, biochemical characteristics, and hormonal status of barley plants and the content of petroleum hydrocarbons in the soil. *Plants*, 10(8), Article 1745. https://doi.org/10.3390/plants10081745

Lednev, S. A., Semenkov, I. N., Klink, G. V., Krechetov, P. P., Sharapova, A. V., & Koroleva, T. V. (2021). Impact of kerosene pollution on ground vegetation of southern taiga in the Amur Region, Russia. *Science of The Total Environment,* 772, Article 144965. https://doi.org/10.1016/j.scitotenv.2021.144965

Liwanag, E. J., Dulay, R. M., & Kalaw, S. (2020). Mycelial growth of Philippine mushroom *Lentinus tigrinus* in selected cucurbit-based media and its antioxidant activity. *Asian Journal of Agriculture and Biology*, 8(3), 323-329. https://doi.org/10.35495/ajab.2019.12.577

Mittal, A., & Singh, P. (2009). Isolation of hydrocarbon degrading bacteria from soils contaminated with crude oil spills. *Indian Journal of Experimental Biology*, 47(9), 760-765.

Mohammed, S. A., Zrary, T. J. O., & Hasan, A. H. (2023). Degradation of crude oil and the pure hydrocarbon fractions by indigenous soil microorganisms. *Biologia*, 78(12), 3637-3651. https://doi.org/10.1007/s11756-023-01513-4

Nei, M., & Kumar, S. (2000). *Molecular evolution and phylogenetics*. Oxford University Press.

Oudot, J. (1984). Rates of microbial degradation of petroleum components as determined by computerized capillary gas chromatography and computerized mass spectrometry. *Marine Environmental Research*, 13(4), 277-302.

Phillips, R. (2013). *Mushrooms: A comprehensive guide to mushroom identification*. Pan Macmillan.

Phonemany, M., Raghoonundon, B., Luangharn, T., Tang, S. M., Hyde, K. D., & Thongklang, N. (2021). A mini review on the potential pharmacological properties, cultivation, and market value of edible Lentinus mushrooms (Polyporaceae). *Fungal Biotec*, 1(2), 49-64. https://doi.org/10.5943/FunBiotec/1/2/4

Ragasa, C. Y., Tan, M. C. S., De Castro, M. E., De Los Reyes, M. M., Oyong, G. G., & Shen, C.-C. (2018). Sterols from *Lentinus tigrinus. Pharmacognosy Journal*, 10(6), 1079-1081. https://doi.org/10.5530/pj.2018.6.182

Raju, M. N., & Scalvenzi, L. (2017). Petroleum degradation: Promising biotechnological tools for bioremediation. In M. Zoveidavianpoor (Ed.). *Recent insights in petroleum science and engineering*. (pp. 351-368) IntechOpen. https://doi.org/10.5772/intechopen.70109

Rzhetsky, A. & Nei, M. (1992). A simple method for estimating and testing minimum evolution trees. *Molecular Biology and Evolution*, 9, 945-967.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, 4, 406-425.

Senthilarasu, G. (2015). The lentinoid fungi (*Lentinus* and *Panus*) from Western ghats, India. *IMA fungus*, 6, 119-128. https://doi.org/10.5598/imafungus.2015.06.01.06

Suliaman, S. Q., AL- Khesraji, T. O., & Hassan, A. A. (2017). New records of basidiomycetous macrofugi from Kurdistan region- Northern Iraq. *African Journal of Plant Science*, 11(6), 209-219. https://doi.org/10.5897/AJPS2017.1543

Tamura, K., Nei, M., & Kumar, S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences*, 101, 11030-11035. https://doi.org/10.1073/pnas.0404206101

Thouand, G., Bauda, P., Oudot, J., Kirsch, G., Sutton, C., & Vidalie, J. F. (1999). Laboratory evaluation of crude oil biodegradation with commercial or natural microbial inocula. *Canadian Journal of Microbiology*, 45(2), 106-115. https://doi.org/10.1139/w98-210

Tomer, A., Singh, R., Singh, S. K., Dwivedi, S. A., Reddy, C. U., Keloth, M. R. A., & Rachel, R. (2021). Role of fungi in bioremediation and environmental sustainability. In R. Prasad, S. C. Nayak, R. N. Kharwar, & N. K. Dubey (Eds.). *Mycoremediation and Environmental Sustainability*. *Volume 3* (pp. 187-200). Springer. https://doi.org/10.1007/978-3-030-54422-5_8

Watanabe, T. (2002). *Pictorial atlas of soil and seed fungi. Morphologies of cultured fungi and key to species*. (2nd ed.) CRC Press.

Zhang, L., Morisaki, H., Wei, Y., Li, Z., Yang, L., Zhou, Q., Zhang, X., Xing, W., Hu, M., Shima, M., Toriba, A., Hayakawa, K., & Tang, N. (2020). $PM_{2.5}$-bound polycyclic aromatic hydrocarbons and nitro-polycyclic aromatic hydrocarbons inside and outside a primary school classroom in Beijing: Concentration, composition, and inhalation cancer risk. *Science of the Total Environment*, 705, Article 135840. https://doi.org/10.1016/j.scitotenv.2019.135840