ISSN 2664-4169 (Print) & ISSN 2664-7923 (Online)
South Asian Research Journal of Biology and Applied Biosciences
Abbreviated Key Title: South Asian Res J Bio Appl Biosci

| Volume-7 | Issue-2 | Mar-Apr -2025 | **DOI: https://doi.org/10.36346/sarjbab.2025.v07i02.007**

Original Research Article

Sustainable Bioremediation of Petroleum Hydrocarbons Using the Edible Mushroom Pleurotus ostreatus in Babylon Province, Iraq

Sarmad Jassem Mohammed[1*]

[1]AL-Qasim Green University, Faculty of Biotechnology, Department of Medical Biotechnology, Iraq

***Corresponding Author:** Sarmad Jassem Mohammed
AL-Qasim Green University, Faculty of Biotechnology, Department of Medical Biotechnology, Iraq

Article History
Received: 16.02.2025
Accepted: 24.03.2025
Published: 08.04.2025

Abstract: ***Background*:** Human health alongside ecosystems face critical dangers because environmental pollution from petroleum products produces rising amounts of polycyclic aromatic hydrocarbons (PAHs). Bioremediation has gained interest as a sustainable removal method because traditional petroleum hydrocarbon removal practices prove both inefficient and expensive. ***Aim*:** The purpose of this study was to assess the growth and adaptability of the oyster mushroom, Pleurotus ostreatus, under different contamination levels in Babylon Province, Iraq, in order to determine its biodegradation potential for petroleum hydrocarbons from used engine oil. ***Methodology*:** Researcher cultivated P. ostreatus samples through laboratory settings using PDA media with three oil concentrations of used engine oil starting at 1% up to 3%. The research tracked the growth rates while observing morphological changes through an 8-day timeframe. The analysis of data determined whether the treatment conditions produced different growth patterns. ***Results*:** At the highest used engine oil concentration (3%) P. ostreatus growth decreased but the microorganism demonstrated adaptive capabilities as inhibition reduced between day one and day eight. Lower concentrations of oil retained stable inoculum viability throughout the period yet viability decreased slightly at high concentrations after 8 days. ***Conclusion*:** The biotechnical potential of P. ostreatus exists as a viable approach for cleaning petroleum pollutants from soil environments. Though aggressive at first growth suppression occurs the fungus demonstrates sufficient adjustability to degrade hydrocarbons which positions it as an effective agent for sustainable bioremediation. Additional investigations into petroleum biodegradation through optimal environmental conditions and real applications will improve its overall effectiveness.

Keywords: *Pleurotus ostreatus*, Biodegradation, Petroleum Hydrocarbons, Used Engine Oil.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) have contaminated the environment to varying degrees due to rising industrialisation and the incorrect disposal of petroleum products. All three of these things—extreme persistence, carcinogenicity, and toxicity—are bad for people and ecosystems [1]. Because to their chemical stability and hydrophobicity, PAHs can accumulate in air, soil, and water; they mainly come from crude oil, petroleum derivatives, and incomplete combustion of organic materials [2]. Remediating areas polluted with PAHs is an issue of international importance since these chemicals remain in the environment. Various ineffective methods to remove petroleum hydrocarbons such as soil washing, chemical oxidation and cremation are commonly used today. The production of secondary pollutants occurs through these methods while their efficiency decreases significantly when used in larger applications and they remain expensive [3, 4]. The adoption of bioremediation as a solution expanded because it presents environmentally friendly possibilities. Scientists have developed an interesting new cleanup technique that uses microbes combined with fungus to decompose and detoxify toxic substances found in contaminated areas [5]. Fungi interest researchers through their production of extracellular ligninolytic enzymes including lignin peroxidase (LiP) manganese peroxidase (MnP) and laccase to help degrade waste using bioremediation strategies. These enzymes possess the ability to biodegrade the most complex hydrocarbon substances [6, 7]. White-rot fungus stands out among biological agents for its applications because it includes Pleurotus ostreatus and other similar species. P. ostreatus functions as a biocontrol

agent against multiple dangerous fungi due to its robust antagonistic features as reported in [8]. Solid substrates show better performance to fungi than bacteria during colonization under harsh conditions due to their superior ability to adapt to unfriendly environments [9]. The wide hyphal networks enable fungi to obtain resources efficiently thus enabling them to survive in low nutritious and dry environments [10]. Slow-growing fungi demonstrate enhanced stress-resistance while showcasing unique shape changes in their environment [11]. Scientific research shows Pleurotus ostreatus (oyster mushroom) should become an important commercial edible fungus because of its strong ligninolytic enzyme activity. The scientific literature shows P. ostreatus has enzymatic capabilities to break down old motor oils alongside diesel and crude oil and numerous other hydrocarbons [12, 13]. In spite of the increasing interest in fungal bioremediation worldwide, very little study has been carried out in areas such as Iraq, where oil exploration, spills, and industrial waste discharge pose a substantial threat to the environment [14]. So, the purpose of this research is to find out how well P. ostreatus biodegrades petroleum hydrocarbons from old motor oil, how well it grows and adapts to varied degrees of contamination, and how it could be utilised to create long-term bioremediation plans.

MATERIAL AND METHODS

The experiments were conducted in Advanced Mycology Lab Department of biology, College of Science, University of Babylon, Iraq.

Source of *Pleurotus ostreatus*

P. ostreatus isolate was obtained from prof. Dr. Jawad K Abood Al-janabi, Advanced Mycology Lab. Department of Biology, College of Science, University of Babylon. Fungal colonies were selected and sub-cultured on Petri plate containing Potato Dextrose Agar (PDA), incubated at 26 ± 2 oC) for 7 days until pure colonies were obtained.

Developing and Sustaining Fungal Isolates

A fungus was introduced into Petri dishes with PDA (pH 7.0), and then let to incubate for 8 days at 26 ± 2 oC. The slants were made by pouring 20 ml of PDA into glass tubes and letting it set until it hardened. The fungal isolates were streaked onto the slants to inoculate them, and then they were incubated at 26 ± 2 oC for 6 days. Subculturing was performed at regular intervals of thirty days after the slants were stored in the refrigerator at a temperature of five degrees Celsius [15].

Growth of *P. ostreatus* under different concentrations of used engine oil

The growth capabilities of *P. ostreatus* in three concentrations of oil-contaminated PDA media {1%, 2% and 3% (v/v)} of used engine oil were investigated, non-oilcontaminated PDA medium was considering as control treatment. Sixteen Petri were prepared and the inoculation was carried out by taking 0.5 cm diameter disc from the edge of the recent colony using sterilized punch-hole tool to cut the mycelium and placed into a center of each plate containing a new sterilized PDA medium by using a transplant needle. All the petri dishes were then incubated under aseptic condition and incubated in the darkness at 25 ± 2 o C. Fungal growth was determined at two-day intervals by measuring the diameter of the colony with a Vernier by taking the average of 2 perpendicular diameter of colony until the Petri dish was completely covered. Four replicates were measured per each concentration [16].

Morphological characteristics of *P. ostreatus*

Visual observation was used to identify the main characteristics of P. ostreatus mycelia, which include texture (cottony or floccose), density (high, regular or low), colour (off-white, white or pale pink), and growth (scarce, regular or abundant), after 15 mL of PDA medium had been used to colonise 10 Petri dishes [17].

2-5 Statistical analysis:

A random complete block design was used to arrange the present tests. Analysis of variance was used to examine the collected quantitative data, and LSD comparisons were performed at the 5% probability level to establish the level of significance.

RESULTS

Morphological characteristics of *P. ostreatus*

In this study, *P. ostreatus* was successfully cultured under laboratory conditions to evaluate the effect of used engine oil on its mycelial growth. The colony morphology varied significantly with oil concentration. The untreated control exhibited a white-milky color with regular, profuse, and fast-growing mycelium (Figure 1A). In contrast, colonies exposed to 1%, 2%, and 3% used engine oil developed a bright cottony appearance, slightly irregular margins, reduced mycelial density, and slower growth (Figure 1B, C, and D).

Figure 1: Colony morphology of *P. ostreatus* isolate at different concentrations: (A) control, (B) 1%, (C) 2%, and (D) 3%, grown on PDA for 8 days after inoculation at 28°C

Effect of Used Engine Oil on the Mycelial Growth of *P. ostreatus*

A one-way ANOVA revealed statistically significant differences ($p < 0.05$) in the growth rates of *P. ostreatus* across different concentrations of used engine oil. The observed mycelial colony diameter varied significantly among the oil-treated groups compared to the control (Table 1). Both oil concentration and incubation time significantly influenced fungal growth over the experimental period ($p < 0.05$).

At 2 days post-inoculation, mycelial growth was highest in the control (2.20 cm), followed by 1% (2.050 cm), 2% (1.825 cm), and 3% (1.725 cm). By 4 days, the control group exhibited a mycelial diameter of 4.725 cm, while the 1%, 2%, and 3% oil treatments resulted in diameters of 4.375 cm, 4.000 cm, and 3.475 cm, respectively. Growth differences continued at 6 days, with diameters of 7.700 cm (control), 6.350 cm (1%), 6.100 cm (2%), and 4.975 cm (3%). Finally, at 8 days, mycelial diameters reached 8.500 cm in the control, 7.600 cm at 1%, 7.325 cm at 2%, and 7.025 cm at 3%. These results demonstrate a significant reduction in growth with increasing oil concentration.

Table 1: Summarizes the effect of used oil concentrations on *P. ostreatus* mycelial growth over the 8-day incubation period at 28°C. Each value is expressed as mean ± standard error (SE)

Used oil %	Time after inoculation (Days)			
	2d	**4d**	**6d**	**8d**
0	**2.20 ± 0.141 b**	**4.725± 0.206 d**	**7.700± 0.141 c**	**8.500± 0.00 c**
1ml	**2.050 ± 0. 10 b**	**4.375 ± 0. 125 c**	**6.350 ± 0. 404 b**	**7.600± 0. 294 b**
2ml	**1. 825 ± 0.095 a**	**4.000 ± 0.163 b**	**6.100 ± 0.182 b**	**. 7.325 ± 0.236 ab**
3ml	**1.725 ± .0957 a**	**3.475 ± 0.221 a**	**4.975 ± 0.670 a**	**7.025 ± 0.464 a**

When comparing the oil-treated groups (1%, 2%, and 3% petroleum oil concentrations) to the control (0%), the results revealed a significant reduction in the growth of *P. ostreatus* on PDA supplemented with petroleum oil. Mycelial growth was reduced by 93.2%, 82.95%, and 78.4% at 2 days, 92.6%, 84.6%, and 76.6% at 4 days, and 82.46%, 79.2%, and 64.6% at 6 days for 1%, 2%, and 3% oil concentrations, respectively. However, by the 8th day, growth inhibition was less pronounced, with reductions of 89.4%, 86.2%, and 82.64% compared to the control (Figure 2).

These results indicate that while petroleum oil initially suppresses fungal growth, *P. ostreatus* demonstrates an adaptive response over time. By the 8th day, its tolerance to petroleum oil increased, suggesting an enhanced biodegradation capability at higher oil concentrations.

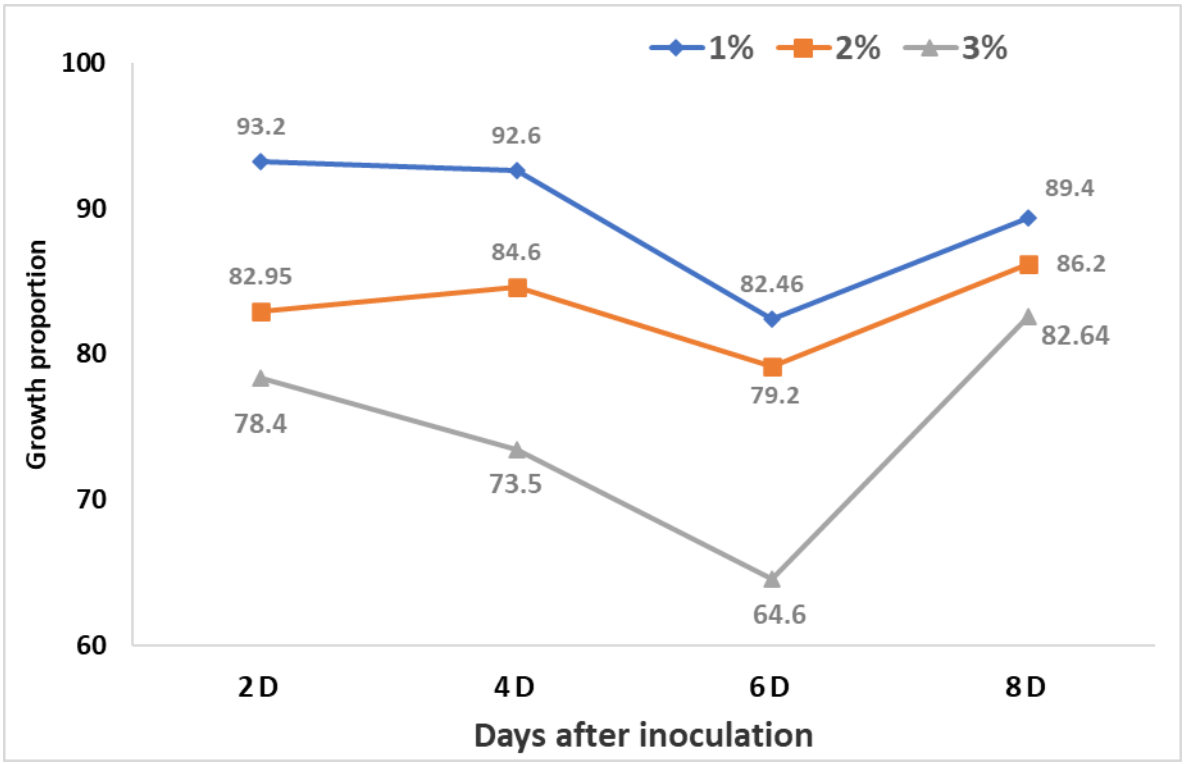

Figure 2: Relative growth of *P. ostreatus* on PDA at 28°C, expressed as a percentage of the control over an 8-day incubation period

Effect of Used Engine Oil on the Inoculum Viability of *P. ostreatus*

The viability of *P. ostreatus* was not significantly affected by used engine oil at any concentration after 2 days of inoculation. However, by day 4, significant differences were observed at 1% and 2% oil concentrations, while no significant difference was detected between the control and 3% treatment. By day 6, no significant differences were found among all treatments compared to the control. In contrast, at day 8, viability was significantly reduced in all oil-treated groups compared to the control, except for the 1% treatment, which remained statistically similar to the control (Fig 3).

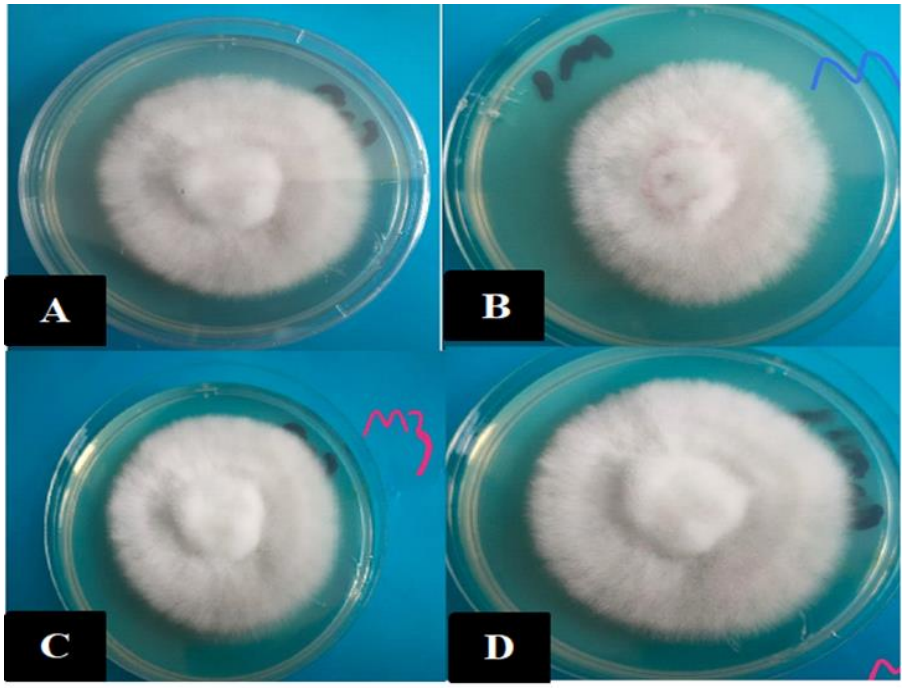

Figure 3: Viability of *P. ostreatus* inoculum at different concentrations of used engine oil: (A) Control, (B) 1%, (C) 2%, and (D) 3%, grown on PDA for 8 days at 28°C

Effect of Used Engine Oil on the Inoculum Viability of *P. ostreatus*

The viability of *P. ostreatus* was not significantly affected by used engine oil at any concentration after 2 days of inoculation. However, after 4 days, significant differences were observed at 1% and 2% oil concentrations, while the 3% treatment remained statistically similar to the control. By day 6, no significant differences were detected among any treatments. In contrast, after 8 days, all oil-treated groups showed significant reductions in viability compared to the control, except for the 1% treatment, which remained statistically similar to the control (Figure 3 and Table 2).

Table 2: Effect of used engine oil concentrations (0, 1, 2, and 3%) in PDA on the inoculum viability of *P. ostreatus* after 8 days of incubation at 28°C. Values are expressed as mean ± standard error (SE)

Used oil %	Time after inoculation			
	2d	**4d**	**6d**	**8d**
0	**2.050±0.191 a**	**5.625±0.478 b**	**7.900±0.294 a**	**8.500± 0.00 c**
1ml	**2.100± 0. 081 a**	**4.95± 0. 1000 a**	**7.700± 0. 244 a**	**8.375± 0. 0500 c**
2ml	**1.975 ± 0.125 a**	**5.050 ± 0.251**	**7.425 ± 0.457 a**	**8.075 ± 0.125 b**
3ml	**2.025 ± 0.170 a**	**5.775 ± 0.382 b**	**7.520 ± 0.377 a**	**7.850 ± 0.129 a**

DISCUSSION

Hydrophobic hydrocarbons, such as those found in petroleum and used engine oil, pose significant environmental hazards due to their toxicity to soil and aquatic microorganisms, including bacteria, fungi, and algae. These hydrocarbons accumulate in cellular membranes, disrupting their integrity and impairing microbial function [18]. One of thea most effective strategies for remediating petroleum-contaminated environments is biodegradation using white-rot fungi, which produce extracellular enzymes capable of breaking down complex hydrocarbons into less toxic byproducts [19, 20]. The bacterial behavior toward petroleum hydrocarbon biodegradation showed significant outcomes from Pleurotus ostreatus while growing on PDA medium containing different amounts of used engine oil for eight days. As P. ostreatus adapted over time at day 8 it indicated that the fungus could degrade hydrocarbons even when growth decreased with rising oil concentration levels. The petroleum degrading process was likely enabled by mycelium which converted long-chain hydrocarbons into shorter chain compounds and transformed polycyclic aromatic hydrocarbons (PAHs) into intermediate byproducts [21]. P. ostreatus shows a high biodegradation efficiency because it produces the ligninolytic enzymes lignin peroxidase, manganese peroxidase, and laccases. The enzymes function as vital agents in hydrocarbon fraction breakdown which leads to both crude oil fluidity reduction and toxicity diminution [22]. The breakdown of high-molecular-weight hydrocarbons depends on laccase activity because it allows the fungus to decompose compounds that exist outside the cell wall [23].

The activity levels of P. ostreatus laccase depend on both temperature and solution pH value. The most productive conditions for laccase production occurred at 25°C (24) but maximum enzyme activity exhibited itself at 30°C and pH of 3 [24]. Laboratory studies have proven P. ostreatus laccase works effectively against hydrocarbons and phenols and textile dyes according to report [25]. Research results showed that Pleurotus ostreatus could survive at all oil concentrations up to 3% in the experimental setting. P. ostreatus displays promising potential to function as an environmentally friendly bioremediation tool for petroleum-spilled sites in the environment. The real-world application of bioremediation using fungi includes agricultural fields and industrial waste management areas and oil spill recovery sites because the inoculation of suitable fungal strains improves the degradation of pollutants and supports soil recovery.

The future research should analyze how different environmental factors such as pH, salinity, oxygen levels and nutrient availability affect P. ostreatus's ability to degrade hydrocarbons [26]. The combination of P. ostreatus with hydrocarbon-degrading bacteria through co-culture could speed up degradation processes while making it suitable for various contaminated sites [27]. The combination of fungal-based bioremediation with microbial consortia shows potential as an effective method to deal with petroleum pollution.

CONCLUSION

Research findings demonstrate Pleurotus ostreatus stands as an effective biological agent for petroleum-contaminated environment cleanup work. The fungus maintained strong adaptability to higher oil concentrations along with minimal growth patterns that suggest its enzymatic breakdown of petroleum hydrocarbons. P. ostreatus shows a dual advantage during 3% soil contamination as it efficiently breaks down pollutants while keeping its cells alive which makes the fungus effective for polluted soil remediation works. P. ostreatus offers an environmentally friendly economical alternative for petroleum pollution remediation because modern remediation strategies need sustainable solutions. Research must identify optimal environmental settings for degradation effectiveness while monitoring P. ostreatus biodegrading potential at real polluted sites together with investigations of its coordination with other microbial degraders. Having fungal bioremediation systems implemented would establish an innovative oil contamination solution that protects our environment while promoting agricultural sustainability.

ACKNOWLEDGMENT

I extend thankful appreciation to the Department of Biology at the College of Science within University of Babylon for their provision of research facilities which enabled the successful execution of my scientific investigation. I am deeply grateful to Professor Jawad K. Abood Al-Janabi who gave his important scientific guidance as well as supplied the Pleurotus ostreatus isolate for this research.

REFERENCES

1. Abdel-Shafy, H. I., & Mansour, M. S. M. (2016). A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health, and remediation. *Egyptian Journal of Petroleum*, 25(1), 107–123.
2. Liu, H., Liu, J., & Zhang, Z. (2018). Bioremediation of polycyclic aromatic hydrocarbons (PAHs) contaminated soils. *Frontiers in Microbiology*, 9, 1283.
3. Gan, S., Lau, E. V., & Ng, H. K. (2009). Remediation of soils contaminated with polycyclic aromatic hydrocarbons (PAHs). Journal of Hazardous Materials, 172(2–3), 532–549. https://doi.org/10.1016/j.jhazmat.2009.07.118
4. Bui, X. T., Vo, T. P. T., Ngo, H. H., Guo, W. S., & Nguyen, T. T. (2016). Multicriteria assessment of advanced treatment technologies for micropollutants removal at large-scale applications. Science of the Total Environment, 563, 1050-1067.
5. Thirumalaivasan, N., Gnanasekaran, L., Kumar, S., Durvasulu, R., Sundaram, T., Rajendran, S., ... & Kanagaraj, K. (2024). Utilization of fungal and bacterial bioremediation techniques for the treatment of toxic waste and biowaste. *Frontiers in Materials*, *11*, 1416445.
6. Bamforth, S. M., & Singleton, I. (2005). Bioremediation of polycyclic aromatic hydrocarbons: Current knowledge and future directions. *Journal of Chemical Technology & Biotechnology*, 80(7), 723–736. https://doi.org/10.1002/jctb.1276
7. Harms, H., Schlosser, D., & Wick, L. Y. (2011). Untapped potential: Exploiting fungi in bioremediation of hazardous chemicals. *Nature Reviews Microbiology*, 9(3), 177–192. https://doi.org/10.1038/nrmicro2519
8. Husseina, N. A., Al-Janabib, H. J., Al-Mashhadyc, F. R., Al-Janabia, J. K. A., & Al-Shujairia, A. R. S. (2022). Antagonistic activities of bioagent fungi Trichoderma harzianum and Pleurotus ostreatus against three species of Fusarium in cucumber plants. *Asia Pacific Journal of Molecular Biology and Biotechnology*, *30*(1), 12-21.
9. Coleine, C., Stajich, J. E., & Selbmann, L. (2022). Fungi are key players in extreme ecosystems. *Trends in ecology & evolution*, *37*(6), 517-528.
10. Heaton, L. L., Jones, N. S., & Fricker, M. D. (2020). A mechanistic explanation of the transition to simple multicellularity in fungi. *Nature communications*, *11*(1), 2594.
11. Gostinčar, C., Zalar, P., & Gunde-Cimerman, N. (2022). No need for speed: slow development of fungi in extreme environments. *Fungal Biology Reviews*, *39*, 1-14.
12. García-Delgado, C., Alfaro-Barta, A., & Eymar, E. (2015). The ability of *Pleurotus ostreatus* to remove emerging pollutants (naproxen and carbamazepine) and its effect on the fungal ligninolytic enzymatic system. *Environmental Science and Pollution Research*, 22(20), 16077–16091. https://doi.org/10.1007/s11356-015-4794-3
13. Hassan, A. R., Kim, K. R., Joo, J. H., & Choi, J. H. (2020). Potential of white-rot fungi to treat petroleum-contaminated soil in cold regions: A review. *Environmental Technology & Innovation*, 18, 100760. https://doi.org/10.1016/j.eti.2020.100760
14. Al-Saad, H. T., Al-Taein, S. M., & Al-Hello, M. A. (2016). Assessment of petroleum hydrocarbons pollution in water and sediments of Shatt Al-Arab River, Basrah, Iraq. *Marsh Bulletin*, 11(1), 65–79.
15. Obaid, A. J., Abood Al-Janabi, J. K., & Taj-Aldin, W. R. (2020). Bioactivities of Anethole, Astragalin and Cryptochlorogenic Acid Extracted from Anise Oil and Moringa oleifera on the Keratinase Gene Expression of Trichophyton rubrum. *Journal of Pure & Applied Microbiology*, *14*(1).
16. Khazia, M. Q., & Al-Janabi, J. K. A. (2019). Antifungal activity of combination of medicinal plant extracts with terbinafine through regulating subtilisin virulence genes in Microsporum canis. *Drug Invention Today*, *12*(11).
17. Sobal, M., Mata, G., & Gaitán-Hernández, R. (2007). Classical characterization of mushroom genetic resources from temperate and tropical regions of Mexico. Micol. Apl. Int., 19(1), 15-23.
18. Alaidaroos, B. A. (2023). Advancing eco-sustainable bioremediation for hydrocarbon contaminants: challenges and solutions. *Processes*, *11*(10), 3036.
19. Burns, R. G., & Wallenstein, M. D. (2010). Microbial extracellular enzymes and natural and synthetic polymer degradation in soil: current research and future prospects. *Gilkes R & N Prakongkep (eds)*.
20. Harms, H., Schlosser, D., & Wick, L. Y. (2011). Untapped potential: Exploiting fungi in bioremediation of hazardous chemicals. *Nature Reviews Microbiology*, 9(3), 177–192.
21. Fallahi, M., Sarempour, M., & Mirzadi Gohari, A. (2023). Potential biodegradation of polycyclic aromatic hydrocarbons (PAHs) and petroleum hydrocarbons by indigenous fungi recovered from crude oil-contaminated soil in Iran. *Scientific Reports*, *13*(1), 22153.
22. Zhang, H., & Zhang, J. (2014). Degradation of polycyclic aromatic hydrocarbons by white-rot fungi. *Environmental Toxicology and Chemistry*, 33(1), 1–9.

23. Alvarez, R., Monje, L., & Díaz, M. (2022). Biodegradation of petroleum hydrocarbons using fungi: A review of enzymatic mechanisms and bioremediation strategies. *Applied Microbiology and Biotechnology*, 106(3), 1135–1152. https://doi.org/10.1007/s00253-022-11893-5
24. Mauricio-Gutiérrez, A., Jiménez-Salgado, T., Tapia-Hernández, A., Cavazos-Arroyo, J., & Pérez-Armendáriz, B. (2014). Biodegradation of hydrocarbons exploiting spent substrate from Pleurotus ostreatus in agricultural soils. *African Journal of Biotechnology*, *13*(33).
25. Johnnie, D. A., Issac, R., Prabha, M. L., & Gomez, L. A. (2023). Biodegradation Assay of heavy metals and Dyes Decolourization in Textile Industrial Effluent using Laccase Isolated from Pleurotus ostreatus. J Pure Appl Microbiol. Published online 21 November 2023. doi: 10.22207/JPAM.
26. Khan, S., Afzal, M., & Iqbal, H. (2022). The role of fungi in petroleum hydrocarbon degradation and their biotechnological applications. *Journal of Environmental Management*, 301, 113923. https://doi.org/10.1016/j.jenvman.2021.113923
27. Johnnie, D. A., Issac, R., Prabha, M. L., & Gomez, L. A. (2023). Biodegradation Assay of heavy metals and Dyes Decolourization in Textile Industrial Effluent using Laccase Isolated from Pleurotus ostreatus. J Pure Appl Microbiol. Published online