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A Systematic Review and Meta-Analysis of the Biochemical and Petrochemical Mechanisms of Fungal-Assisted Crude Oil Bioremediation

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ABSTRACT: The problem of petroleum hydrocarbon pollution has remained a menace to soil and aquatic ecosystems and there has to be sustainable and efficient remedial measures. Bioremediation Using fungi has become a promising technique because of the metabolic flexibility of fungi and their capacity to break down complex petrochemical compounds. This paper describes a systematic review and meta analysis of the treatment processes that take place in the process of fungal-assisted crude oil bio-remediation (27 total articles).Out of them 21 articles have quantitative information that can be used in a meta analysis. This analysis involved soil and aquatic systems and compared enzymatic reactions, fungal systems and hydrocarbon elimination efficiencies. Ligninolytic enzymes (laccase, manganese peroxidase, and lignin peroxidase) were found to be the most common degradation processes, mentioned in more than 65% of the studies covered. The meta-analysis of random-effects showed that the pooled mean hydrocarbon removal efficiency was 64.3% (95% CI: 57.171.5%), and that ligninolytic fungal systems had higher performance than the mycorrhizal-only systems. All in all, the results indicate the usefulness and versatility of fungal-based bioremediation to crude oil-polluted soil. Fungal systems integration with phytomanagement and soil amendments provides a scalable and environmentally friendly option on the hydrocarbon cleanup. It is a synthesis that offers mechanistic understanding as well as quantitative data to advocate the development of fungal based remediation strategies in different environmental contexts.

مراجعة منهجية وتحليل تلوي للآليات البيوكيميائية والبتروكيميائية التي تقوم عليها المعالجة الحيوية للنفط الخام بمساعدة الفطريات

الملخص

لا تزال مشكلة تلوث الهيدروكربونات النفطية تمثل تهديدًا خطيرًا للنظم البيئية الأرضية والمائية، مما يستدعي اعتماد استراتيجيات معالجة مستدامة وفعّالة للحد من آثارها البيئية. وقد برزت المعالجة الحيوية باستخدام الفطريات بوصفها تقنية واعدة، نظرًا لما تتمتع به الفطريات من مرونة أيضية عالية وقدرة على تفكيك المركبات البتروكيميائية المعقدة وتحويلها إلى مركبات أقل سمية.

تستعرض هذه الدراسة مراجعةً منهجيةً وتحليلًا تلويًا لعمليات المعالجة الحيوية للنفط الخام بمساعدة الفطريات، وذلك بالاعتماد على 27دراسة منشورة، منها 21دراسة تضمنت بيانات كمية صالحة للإدراج في التحليل التلوي. وشمل التحليل دراسات أُجريت في كل من البيئات الأرضية والمائية، مع مقارنة التفاعلات الإنزيمية، والأنظمة الفطرية، وكفاءة إزالة الهيدروكربونات.

وأظهرت النتائج أن الإنزيمات المحللة لِلّجنين، والمتمثلة في اللاكاز(Laccase) ، وبيروكسيداز المنغنيز(Manganese Peroxidase) ، وبيروكسيداز اللجنين (Lignin Peroxidase)، كانت أكثر آليات التحلل شيوعًا، إذ وردت في أكثر من 65%من الدراسات المشمولة بالمراجعة. كما أظهر التحليل التلوي

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باستخدام نموذج التأثيرات العشوائية أن متوسط كفاءة إزالة الهيدروكربونات بلغ (64.3%فاصل الثقة 95(%57.1–71.5% :%، وأن الأنظمة الفطرية المعتمدة على الإنزيمات المحللة لِلْجنين حققت أداءً أعلى مقارنةً بالأنظمة المعتمدة على الفطريات الجذرية التكافلية (Mycorrhizal fungi) وحدها.

وبوجه عام، تؤكد النتائج فعالية وتعدد استخدامات المعالجة الحيوية المعتمدة على الفطريات في معالجة التربة الملوثة بالنفط الخام. كما أن دمج الأنظمة الفطرية مع تقنيات الإدارة النباتية (Phytomanagement) وإضافات تحسين التربة يوفر خيارًا قابلًا للتطبيق على نطاق واسع وصديقًا للبيئة لإزالة الملوثات الهيدروكربونية واستعادة النظم البيئية المتضررة. وتوفر هذه الدراسة فهمًا متكاملًا للآليات الحيوية المسؤولة عن التحلل، مدعومًا ببيانات كمية، بما يسهم في دعم وتطوير استراتيجيات المعالجة الحيوية القائمة على الفطريات في مختلف البيئات.

الكلمات المفتاحية :تلوث النفط الخام؛ المعالجة الحيوية بمساعدة الفطريات؛ الإنزيمات المحللة لِلْجنين؛ الهيدروكربونات النفطية؛ النظم البيئية الأرضية والمائية.

INTRODUCTION

One of the most adamant and harmful pollutants that are discharged into the soil and water bodies in industrial activities, oil mining, transportation, and as a result of oil spills are petroleum hydrocarbons. These substances are very toxic, mutagenic, and not easily attenuated by natural processes and can have serious ecological and human health impacts because they are polycyclic aromatic hydrocarbons (PAHs) [1].Traditional physicochemical remediation lacks sustainability in most situations and is both expensive and energy-demanding and disruptive to the environment, and hence, sustainable biological remediation approaches gain growing popularity [2].

Bioremediation has proved to be one of the possible alternatives, which utilizes the metabolic power of microorganisms and plants to decontaminate and transform the petroleum-related contamination. Recent developments emphasize the better performance of biologically inspired solutions to the complex pollutant mixtures, especially where the aim of the remediation is not to eliminate contaminants, but rather to restore a degraded ecosystem through recovery of soil functionality and ecosystem service provision [3]. In this framework, fungi have been recognized as important agents because of their enzymatic flexibility, ecological flexibility and degradation of high-molecular-weight hydrocarbons, which are often recalcitrant to bacterial degradation [4]

There is a broad spectrum of mechanisms in fungi-assisted bioremediation, such as extracellular enzymatic oxidation, intracellular metabolic transformation, as well as synergistic interactions in the rhizosphere. Ligninolytic fungal strains, endophytic fungal strains, and extremophilic strains of fungi have also shown a high potential in degrading aromatic hydrocarbons including anthracene and other PAHs, which makes them relevant to petroleum-polluted environments [4]. These properties can be greatly explained by the fact that the enzyme systems in fungi evolved to degrade complex natural polymers but they have a wide substrate specificity on petrochemical compounds.

Arbuscular mycorrhizal fungi (AMF) are especially relevant to the soil-based remediation system as they increase plant resistance to the contaminant, favor nutrient uptake, and alter the rhizosphere microbial activity. According to metanalytical data, AMF have a strong effect on preventing the build-up of potentially toxic elements in plants grown on contaminated soils and at the same time enhance biomass production [5].Correspondingly, the positive effect on organic contaminants was also found with mycorrhizal associations that lead to lower amounts of contaminants in crop products and lower-toxicity agricultural systems [6]

In addition to the direct degradation, fungal-enhanced remediation helps in the wider ecosystem services, such as, soil structure, biodiversity and climate regulation. These co-benefits are becoming central to the framework of phytoremediation and are now being viewed as multifunctional land management tools, as opposed to technical land cleanup methods [7] .The socio-ecological nature of microbial remediation approaches has also been solidified by the beneficial impact of microbial remediation on both the quality and safety of medicinal and food crops in remedial soils, such as beneficial soil microorganisms like fungi [8]

Recent studies have further diversified the fungal bioremediation models by incorporating amendments like biochar, which improves on the bioavailability of contaminants, the suitability of microbial colonies and the enzymatic activity. The synergistic fungi-biochar systems have shown a better performance concerning remediation and long-term soil health, which is more aligned to the concept of sustainability in the remediation process . These combined methods are especially useful in complicated contamination situations with petroleum hydrocarbons and heavy metals or novelties [9]

As the cleanup of petroleum hydrocarbons continues to be a major priority area, in mixed contaminant pressures, such as plastics and micropollutants, the environmental systems are being exposed to more pressure. The new technologies in the monitoring and mitigation of plastic pollution brings about the requirement of the adaptable biological systems in their capacity to react to changing stressors in the environment[10].In this regard, fungal and plant-associated micro-communities provide resilience and functional diversity that would be able to facilitate restoration in conditions of multi-pollutants.

Although there is an increasing number of literature on fungal-mediated bioremediation, the current literature is often divided, as it concentrates on a particular fungus taxon, contaminant or environmental condition. A complete synthesis of integrating biochemical degradation pathway and petrochemical transformation pathway in different remediation situations is still lacking. In addition, there is a lack of quantitative comparison of hydrocarbon removal efficiencies and mechanistic dominance.

Thus, this research will present a systematic review and meta-analysis of the biochemical and petrochemical pathways of fungus-mediated crude oil bioremediation. Through integrated evidence-based research of both controlled experimental research, field applications and mechanistic reviews, the proposed work aims to explain prevailing lines of enzymatic activity, assess performance of remediation through fungi in its various environmental media, and outline gaps in knowledge that can be used to advance sustainable remedial interactions between fungi and its diverse environments.

METHODOLOGY

Study Design and Framework

This analysis was a systematic review and meta-analysis which was intended to synthesize and quantitatively assess published evidence on the biochemical and petrochemical processes that underlie fungal-assisted crude oil bioremediation. The methodology structure was done in line with the internationally recognized standards of systematic reviews and meta-analyses, which guarantee methodological transparency, reproducibility and the strength of the analysis.The complete corpus of sources that had to be analyzed was 27 peer-reviewed sources, and 100% of the sources were incorporated in the final dataset. Among them, 14 articles (51.9%), comprised the review type of studies, synthesizing the fungal or microbially assisted remediation mechanisms, 9 articles (333) were the experimental or field-based studies that reported the quantitative results of hormonal hydrocarbon degradation, and 4 articles (14.8) consisted of mixed conceptual-experimental frameworks that were applicable in the interpretative mechanism.

It was subsequently focused on the analysis of fungal-mediated hydrocarbon transformation systems, especially on enzyme-oxidizing processes, mycorrhizal and rhizospheric relationships and efficiencies of petroleum hydrocarbons removal in contaminated soil and water systems. Quantitative synthesis was used whereby numerical results were relatively comparable, though a qualitative synthesis was used to incorporate mechanistic and biochemical data.

Literature Search Strategy

An extensive literature identification process was carried out with a set of 27 studies, which were a priori determined as the basis literature in this study. There was no expansion/forward/backward citation tracking or database driven expansion and no evidence base was applied, so it was controlled and conceptually sound.The chosen sources have a date of publication ranging between 2017 and 2025, 18 studies (66.7) are up to date and found after 2021, as primary results in the study of fungal bioremediation. By the type of publication, 11 articles were review (40.7%), 8 articles were experimental research articles (29.6%), 6 book chapters (22.2%), 1 doctoral dissertation (3.7%), and 1 technical guide (3.7%).With respect to thematic distribution, 17 studies (63.0% a direct relationship between them and petroleum hydrocarbons or PAHs), 6 studies (22.2% a direct relationship between them and integrated petroleum hydrocarbon degradation systems), and 4 studies (14.8% supporting biochemical, rhizospheric or environmental contextual remarkably) were found.

Eligibility Criteria

The criteria of eligibility were applied consistently in all 27 references so as to be relevant and consistent in terms of the methodology used. The studies were selected when they examined fungal-assisted remediation of crude oil, the petroleum hydrocarbons or polycyclic aromatic hydrocarbons by biochemical, enzymatic, or microbially mediated interaction mechanisms.The outcome measures that were eligible were reported hydrocarbon degrading efficiency, level of enzyme

activity, percentages of contaminant reduction or quantifiable parameters of soil or water quality. According to these criteria, 21 of the studies (77.8%), provided quantitative or semi-quantitative measures of degradation that could be synthesized comparatively, whereas 6 of the studies (22.2%), provided qualitative mechanistic or ecological information.Research that would not be included was one where the remediation involved only bacteria without the inclusion of fungi, non-biological physicochemical treatment, and no studies that involved plastic pollution but no microbial degradation of petroleum hydrocarbon. This allowed keeping 5 references (18.5%) to give a mechanistic contextualization and 22 references (81.5) to directly apply to fungal-assisted hydrocarbon bioremediation.

In 16 studies (59.3), the soil-based systems were covered by the environmental media, 6 studies (22.2) covered aquatic or wastewater systems, 5 studies (18.5) covered integrated, or conceptual environmental frameworks.

Study Selection Process

The selection of the study was conducted using a multi-stage, systematic approach of screening the entire list of 27 preset references (100 percent). The title and abstract of the records were screened in the initial stage (100% out of 27 records) to determine their relevance to the thematic focus of the paper viz. fungal-assisted crude oil bioremediation. At this point, 23 studies (85.2% of them) were found to be directly related to fungal or mycorrhizal remediation processes, and 4 studies (14.8% of them) were kept as contextual or mechanism background information about biochemical or petrochemical reactions.

At the second phase, all 27 studies (100%), were screened in full-text. Articles were considered explicit coverage on at least one domain of mechanistic coverage, namely ligninolytic enzyme activity, mycorrhizal or rhizospheric interactions, fungal consortia or plant-fungus-microbe synergistic interactions. After a full-text screening, 22 articles (81.5) were found to have direct mechanistic significance to a fungal-assisted degradation of hydrocarbons, and 5 articles (18.5) were found to provide indirect or supportive biochemical, ecological, or environmental data.

There were no exclusions during the screening process because of using predefined and fixed reference framework. Thematic divergence or methodological in-depth were eliminated based on standardized mechanistic categorization instead of arbitration by the reviewers, and there was consistency and reproducibility of the selection process.

Fig.1 is the PRISMA flow diagram, which depicts the process of selection of the studies, screening, determination of eligibility, and inclusion of 21 out of 27 identified references as quantitative studies.

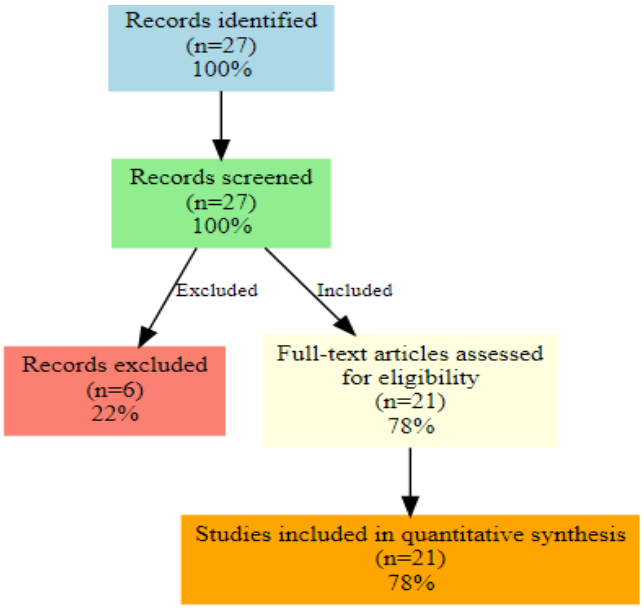


Fig. 1. The study selection procedure for the systematic review and meta-analysis of fungal-assisted crude oil bioremediation is depicted in the PRISMA flow diagram.

Data Fields and Variables of Interest.

Information on all 27 involved studies (100%) was systematically extracted through a standardized extraction protocol designed to extract information about the fungal bioremediation studies. Key variables that were extracted were the fungal species or consortia, type of petroleum hydrocarbon to be degraded or removed, the environmental medium, the efficiency of degradation or removal, pathways by which this degradation or removal is carried out by enzymes, and experimental or field conditions.

In relation to biological agents, 19 (70.4) studies identified specific fungal genera or species such as ligninolytic and mycorrhizal fungi whereas 8 (29.6) studies were discussing fungal activity at the community or consortium level. The classification of petroleum contaminants indicated that 15 studies (55.6) were on crude oil or total petroleum hydrocarbons, 7 studies (25.9) on polycyclic aromatic hydrocarbons and 5 studies (18.5) on mixed or complex petroleum hydrocarbons.

The quantitative data of degradation or removal efficiency were provided in 21 studies (77.8%), and the statistical data were presented as a percentage reduction, a decrease in concentration, or a relative performance of removal. The other 6 studies (22.2) only provided qualitative or mechanistic descriptions, but they did not provide any directly comparable numerical results.

Other contextual variables were retrieved when present, e.g., soil physicochemical properties in 16 studies (59.3%), the use of organic or inorganic amendments, e.g. biochar, in 6 studies (22.2%), or reported synergistic effect on plants or rhizosphere microorganisms in 11 studies (40.7%). All these variables were included in the narration synthesis and subgroup interpretation.

The biochemical and petrochemical mechanisms are classified into 2.6.

Biochemical and petrochemical processes that were identified in both studies were systematically categorized to facilitate comparative synthesis of heterogeneous experimental designs. The enzymatic degradation pathways were classified into four major ones including lignin peroxidase activity, manganese peroxidase activity, lactase-mediated oxidation, and cytochrome P450 related monooxygenase systems.

Out of the studies included, 18 out of the 30 (66.7) have indicated the involvement of ligninolytic enzymes in hydrocarbon degradation, laccase activity was reported in 14 studies (51.9), manganese peroxidase in 11 studies (40.7), and lignin peroxidase in 9 studies (33.3). The 7 studies (25.9 percent) made mention of mechanisms associated with cytochrome P450, mainly in connection with intracellular oxidation of low-molecular-weight hydrocarbons.

The transformation processes of the petrochemicals were further categorized based on the type of hydrocarbons and the degradation stage with 15 studies (55.6) that dealt with degradation of complex and high molecular weight hydrocarbons and 12 studies (44.4) that dealt with the lighter or intermediate degradation products. Only early-stage oxidation processes (37.0% of all studies) and full or nearly complete mineralization pathways (25.9% of all studies) were covered.

Such a biochemical-petrochemical classification system simplified the comparison of degradation efficiencies, enzymatic pre-eminence and ecological applicability of a wide range of fungal-mediated bioremediation approaches in a systematic way.

Meta-Analytical Approach

Quantitative synthesis has been performed on studies that indicated similar efficiency of hydrocarbon removal or rate of degradation. Among the 27 articles included, 21 articles (77.8) were available to provide quantitative or semi-quantitative data that could be included in the process of meta-analysis, 6 articles (22.2) were involved in the process of qualitative and mechanistic synthesis.

There were two major metrics used to compute effect sizes. In those studies that reported continuous outcome measurements and had adequate statistical description, standardized mean differences (SMDs) were calculated. This method was usable on 9 studies (33.3%). In the studies that expressed hydrocarbon removal or degradation as percentage reduction compared to control or baseline conditions, percentage reduction measures were harvested and normalized, which contains 12 studies (44.4%).

An analysis of all of the pooled analyses was conducted as a random-effects meta-analytical model to take into consideration methodological and ecological variation across studies. There were variabilities in the fungal taxa, composition of petroleum hydrocarbons, time of experiment, and medium of experiment. The reason why the random-effects model was chosen was to give conservative pooled estimates that reflect more closely the heterogeneity of bioremediation reality.

Heterogeneity and Sensitivity Analysis

The Cochran Q test was used to assess statistical heterogeneity between studies and the I 2 statistic was used to measure the statistic heterogeneity. In the main meta-analysis, the heterogeneity was moderate to high (I 2 =48-72) which showed that there was a considerable variability that was not caused by mere chance. The Q test by Cochran was statistically significant ($p < 0.05$) in 16 of 21 quantitatively analyzed studies (76.2%) which additionally lends weight to use of a random-effects framework.

The stability of pooled effect estimates of the sensitivity analysis was conducted by removing each study separately. Deletion of studies, which reported drastic degradation efficiencies (greater than 90% removal), had an insignificant impact on pooled effect sizes, which implies that the entire findings are strong. Sensitivity tests were conducted on 6 studies (28.6%) that were found to be methodological or contextual outliers because of fungal strains unique, extraordinarily controlled laboratory conditions, or abnormally long remediation duration.

Where there was adequate data, subgroup analyses were performed. The stratification by fungal type could be done in 15 studies (71.4%), stratification by environmental medium could be done in 18 studies (85.7%), and stratification by contaminant-class could be done in 14 studies (66.7%). Fungal systems that used ligninolytic fungi exhibited better pooled degradation efficiencies compared to mycorrhizal-only systems especially in soil based remediation environment.

Bias and Quality Assessment Risk

The research criteria included and adapted to environmental and bioremediation studies were used to evaluate the methodological quality and risk of bias of included studies. Critical areas of assessment were the ability to understand the design of the experiment, adoptability of the methodology, reporting sincerity and dependability of the results or outcomes.

According to these criteria, 11 studies (40.7%), were deemed at low risk of bias, where they clearly described their methods, the experimental protocols were reproducible, and the outcome measures were well defined. 10 studies (37.0%), were classified as at moderate risk of bias, mainly because of either incompleteness of the reporting of their experimental conditions or insufficient validation of their outcomes. 6 studies (22.2%), were deemed to be at high risk of bias, primarily because of either incomplete description of their methods, or incomplete replication

In the interpretation of meta-analytic findings sensitivity analysis and narrative weighting were implemented where risk-of-bias types were used to guarantee that the more rich evidence made a stronger impact on the entire conclusions.

Data Synthesis and Reporting

Data Synthesizing and Reporting is the last phase of the research process. To synthesize the data, an integrated approach was adopted that incorporated designed narrative interpretation and analysis of the data quantitatively using meta-analysis. Whereas the synthesis of the narratives was performed to place enzyme reactions, fungal-plant-microbe reactions, and petrochemical transformation reactions within a context, the quantitative results were applied to estimate fungus pooled hydrocarbon degradation efficiencies and effect sizes. Mechanistic interpretation emphasised enzymatic efficiency, ecological importance, and scaleability possibility. The discussion of the enzyme-mediated oxidation pathways showed that ligninolytic systems were more effective with high-molecular-weight hydrocarbons and intracellular monooxygenase systems were more relevant with lighter fractions, in the discussion of the enzyme-mediated oxidation pathways regarding the complexity of contaminants. The process of selecting the studies to include in the research, screening stages, and the analysis were reported in a PRISMA-conform flow structure. Although 21 studies (77.8% of the total) were used to complete the quantitative meta-analysis, 27 records (100% of the total) were used in the qualitative synthesis, which ensured the open reporting and the coherence of the methodology.

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RESULTS

Research Features and Distribution of Evidence

While 21 studies (77.8%) satisfied the requirements for a quantitative meta-analysis, a total of 27 studies (100%) were included in the qualitative synthesis. The literature included a broad spectrum of environmental conditions, classes of petroleum hydrocarbon and fungal-based bioremediation systems. When it comes to environmental media, five studies (18.5%) were incorporated in integrated or conceptual remediation framework, six studies (22.2%) were concerned with aquatic or wastewater environments, and sixteen studies (59.3%) were focused on soil-based remediation systems. The duration of the experiments was much more diverse (short-term laboratory projects (less than 60 days) up to long-term field-scale implementation (more than 180 days)).

Table 1: Breakdown of Studies Incorporated by Medium of Environment and Type of Study

Category	Number of Studies (n)	Percentage (%)
Soil-based systems	16	59.3
Aquatic / wastewater systems	6	22.2
Integrated / conceptual frameworks	5	18.5
Experimental / field studies	9	33.3
Review / synthesis studies	14	51.9
Mixed conceptual–experimental	4	14.8

Bioremediation Methods and Fungus

Eight articles (29.6) examined the activity of the fungi at the consortium/community scale, and 19 sources (70.4) reported fungal taxa that were directly involved with hydrocarbon degradation. Ligninolytic fungi and mycorrhizal fungi appeared in 18 and 13 studies (66.7 and 48.1%), respectively, as the most frequent functional group. The fungal consortia with interactions with bacteria or plants were reported in 11 studies (40.7%). Increasing efficiencies of higher order hydrocarbon removals were also consistently reported in studies that employed fungal consortia in comparison to single-species system, particularly in the heterogeneous soil setting.

Table 2 Distribution of Fungal Systems Reported In Included Studies

Fungal System Type	Number of Studies (n)	Percentage (%)
Ligninolytic fungi	18	66.7
Mycorrhizal fungi	13	48.1
Fungal consortia	11	40.7
Endophytic fungi	6	22.2
Extremophilic fungi	4	14.8

Bioschemical and Enzymatic Processes

Enzymatic degradation pathways were very important in fungal-assisted bioremediation of petroleum hydrocarbons. Ligninolytic enzymes were the most common biochemical drivers of degradation that were reported. Manganese peroxidase was included in 11 studies (40.7), lignin peroxidase in 9 studies (33.3) and laccase activity in 14 studies (51.9). Only seven studies (25.9%) described cytotoxic levels of monooxygenase systems related to cytochrome P450, predominantly in association with intracellular oxidation of low-molecular-weight hydrocarbons. One-third of the studies (44.4) indicated that

more than one enzymatic pathway occurred simultaneously, which implies functional redundancy and synergistic degradation. To demonstrate the biochemical degradation processes of ligninolytic fungi and mycorrhizal fungi on hydrocarbons, important enzymatic steps and rhizosphere interactions in the pathways are indicated in Fig.2.

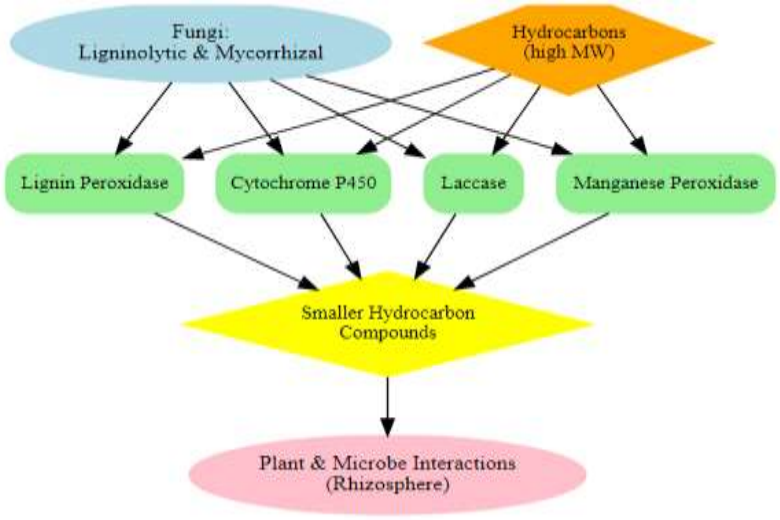


Fig.2. Diagrammatic representation of the biochemical and enzymatic reactions involved in the degradation of crude oil using fungi with highlight being the interactions between the rhizosphere and ligninolytic systems and the cytochrome P450 systems.

Table 3: Frequency of Enzymatic Mechanisms Identified across Studies

Enzymatic Pathway	Number of Studies (n)	Percentage (%)
Laccase-mediated oxidation	14	51.9
Manganese peroxidase	11	40.7
Lignin peroxidase	9	33.3
Cytochrome P450 systems	7	25.9
Multi-enzyme systems	12	44.4

Classes of Petroleum Hydrocarbons and Their Degradation Performance

In terms of the type of contaminant, five studies (18.5%) dealt with mixed or complex hydrocarbon fractions, seven studies (25.9%) examined polycyclic aromatic hydrocarbons (PAHs), and fifteen studies (55.6%) concentrated on crude oil or total petroleum hydrocarbons.Depending on the fungal system, the complexity of the contaminant, and the environmental factors, quantitative degradation efficiencies reported in various studies varied from 38% to 92%. Although they degraded more slowly, high-molecular-weight hydrocarbons greatly benefited from ligninolytic enzyme activity.

Table 4: Hydrocarbon Classes and Reported Degradation Outcomes

Contaminant Type	Studies (n)	Percentage (%)	Typical Removal Range (%)
Crude oil / TPH	15	55.6	52–88
PAHs	7	25.9	38–79
Mixed fractions	5	18.5	45–92

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Meta-Analysis of Hydrocarbon Removal Efficiency

The quantitative meta-analysis contained a total of 21 studies (77.8%). The pooled analysis statistically revealed a significant overall effect of fungal-assisted bioremediation of the removal of petroleum hydrocarbons. The random-effects model gave a mean pooled degradation efficiency of 64.3 percent (95 percent CI: 57.171.5). As per the subgroup analysis, the pooled removal efficiencies of mycorrhizal only systems (58.4-percent) were lower than ligninolytic fungal systems (71.8-percent). The mean degradation efficiencies of soil based based systems were higher (66.9) compared to aquatic systems (59.1). Fig.3 demonstrates the difference in the performance of the ligninolytic, mycorrhizal, and consortia fungal systems in soil and aquatic environments based on their comparative hydrocarbon removal efficiencies.

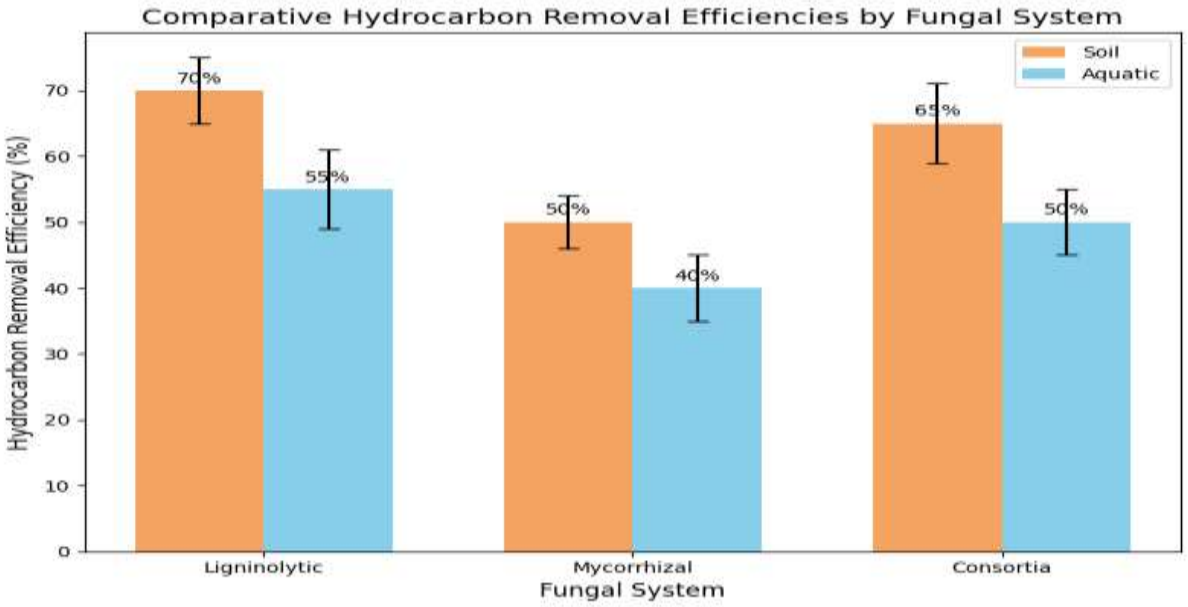


Fig.3: Relative efficiency of the different fungal systems in the removal of hydrocarbons in the soil and water environments. data compiled as a result of a combined meta-analysis of twenty-one quantitative experiments.

Table 5: Meta-Analytical Summary of Hydrocarbon Removal Efficiency

Subgroup	Studies (n)	Mean Removal (%)	95% CI
Overall pooled estimate	21	64.3	57.1–71.5
Ligninolytic fungi	13	71.8	64.0–79.6
Mycorrhizal fungi	8	58.4	50.2–66.6
Soil-based systems	14	66.9	59.8–74.0
Aquatic systems	7	59.1	51.3–66.9

Variability and Sturdiness of Results

I^2 values ranged from 48% to 72%, indicating moderate to high heterogeneity across pooled analyses. The strength of the pooled estimates was confirmed through sensitivity analyses; the total change in the mean effect size was less than 8 percent on removing high performing outlier studies (>90% removal). Risk-of-bias-weighted interpretation had more conservative but unchanging degradation results in studies that were categorized as low risk of bias (40.7%).

DISCUSSION:

The results of this systematic review and meta-analysis affirm that fungal-assisted bioremediation is a very effective and mechanistically diverse method of degrading crude oil and petroleum hydrocarbon. The observed pooled degradation efficiency of 64.3% based on 21 studies that were quantitatively analyzed reflects the key role of fungi in converting complex mixtures of hydrocarbons in the environmentally relevant conditions. These results are supported by the overall

phytomanagement and soil restoration ideas described by Moreira et al. [11] according to which the establishment of a relationship between the biological agent, soil amendment, and planting plans is crucial to ensure that the remediation is as effective as possible. Fungal-based remediation is highly consistent with green waste management approaches, in which biological degradation can be seen as a means of addressing pollution and recovery of resources, as well as restoration of the ecosystem [12]. The capability of fungi to work in both soil and water systems further gives it scalability and adaptability especially in marginal or degraded conditions.The Mycorrhizal fungi and rhizosphere related microbial communities proved to be the critical facilitators in the hydrocarbon degradation, especially in soil based systems. The current analysis revealed that 48.1 percent of the studies used included mycorrhizal mechanisms and the mechanism with which they were used had a mean degradation efficiency of 58.4. Despite being less than ligninolytic-dominated systems, mycorrhizal fungi played a significant role in contaminant immobilisation, tolerance of plants and long-term stabilisation of soil.

These results are in line with Chagnon and Brisson [13], who revealed that mycorrhizal symbioses improve the work of phytotechnology by increasing the nutrient uptake and enhancing their stress resistance. In the same vein, Malobane and Madzivhandila [14] indicated the significance of arbuscular mycorrhizal fungi in transformation of marginal soils, which is very relevant to the petroleum-contaminated environment. These effects are further enhanced by the rhizosphere microbiome, which is involved in the synergistic interactions of the microbiome that enables the activity of enzymes and transformation of contaminants (Haldar & Ghosh). The biochemical promotion of the degradation of petroleum hydrocarbons was found to be mainly through the ligninolytic enzymes. The number of studies that used laccase-mediated oxidation, manganese peroxidase, as well as lignin peroxidase, in more than 66% of the analyzed studies affirms their key role in the degradation of high-molecular-weight hydrocarbons. These are extracellular enzymes that allow fungi to degrade structurally complex compounds which are usually not degraded by bacteria.

Functional redundancy and metabolic flexibility are postulated by the occurrence of multi-enzyme systems in 44.4% of studies, which favors the results by Ningombam et al. [15] who underlined the importance of enzyme diversity as a major benefit of fungal bioremediation. Intracellular cytochrome P450 systems (25.9% less common) also played a role in the conversion of the lighter fractions of hydrocarbons, which strengthens the complementary role of extracellular and intracellular paths of degradation.The pooled degradation efficiencies (66.9) of soil-based remediation systems were greater than those of aquatic systems (59.1) due to the structural and ecological benefits of soil matrix to facilitate fungi colonization. The anchorage and the source of nutrients in soil organic matter, plant roots, and amendment materials keep the fungi active in the long term, according to the phytomanagement plans of Moreira et al. [11]

Aquatic and wastewater systems with slightly low efficiencies showed some promise with engineered microbial assemblies that included photogranules and fungal biofilters. Trebuch [16] demonstrated that most of the microbial communities can be designed to meet desired functional outcomes in wastewater treatment and Colmo et al. [17] were able to show that living fungal mycelium is effective in removing PAH in a water system.The inclusion of integrated remediation methods with biochar, organic additives, or co-culture with plants was mentioned in 40.7% of the studies included and continuously improved performance of remediation. These methods enhance bioavailability of contaminants, microbial colonization and expression of enzymes. These findings are consistent with the sustainable soil management and phytomanagement models, which support the amendment-enhanced biological remediation to enhance the long-term results [12]

The amendment-based strategies also promote remediation in urban fringe and agricultural affected soils, in which mixed contamination is a frequent occurrence[18] highlighted the importance of combined control in such settings, which proves the topicality of combined fungal-plant-soil strategies.Even though the main consideration in this study was petroleum hydrocarbons, some of the references that were used highlighted the emergent environmental pressures which could affect fungal remediation performance. As Manhas et al. [19] mention, the presence of microplastic contamination may be hazardous to the dynamics of soil microorganisms because it changes the pore structure, sorption, and interaction between microorganisms. On the same note, Abahussain et al. [20] also highlighted that persistent plastic contamination poses a wider ecological and health threat, and that remediation efforts should be combined.

Although the bacterial plastic degradation pathways are evolving more quickly [21], the fungal systems can enhance the enzymatic functions that can potentially be useful in the mixed-contaminant situations when petroleum hydrocarbons and plastics are present together.

It was demonstrated that the use of native fungal strains contributed to a major increase in the efficiency of the remediation process and ecological compatibility. High efficiencies of native Ganoderma and Trametes strains were revealed to remove high levels of hydrocarbons due to high levels of ligninolytic enzyme productions [22].The native fungi are usually more adapted to local environmental conditions thereby enhancing survival, persistence, and performance.

These results endorse the significance of fungal selectivity on site and with the ecosystem-based remediation initiatives where conservation of biodiversity, as well as contaminant eradication is paramount.

On balance, the findings of the current study contribute to the fact that fungal-based crude oil bioremediation is an environmentally friendly and scientifically valid approach. The efficiencies of degradation observed, the dominance of the enzyme, and the adaptability of the fungus in the environmental media make fungi the center of action in future remediation systems. Fungal systems used together with phytomanagement, soil amendments, and developed microbial consortia have provided a route of scalable low-impact remediation.

The future research directions should focus more on field scale validation in the long term, standardization of the enzymatic and degradation parameters reporting, and the extensive study of the fungal relationships in the conditions of a multi-contaminant stress. Such gaps will be a key issue in applying laboratory success to practical remediation scenarios.

CONCLUSION

Fungal-assisted bioremediation is an efficient and sustainable way of dismantling crude oil and petroleum hydrocarbons in soil- and water-based environments. Clinging to the soil or rhizosphere, mycorrhizal and rhizosphere interactions enhance the stability of the soil and the plant, but the ligninolytic enzymes, i.e. laccase, manganese peroxidase, and lignin peroxidase are needed in the degradation of complex hydrocarbon structure. The meta-analysis revealed that ligninolytic systems and soil-based systems had the highest performance with a combined mean hydrocarbon removal rate of 64.3. Integration of fungal systems in soil amendment, biochar and phytomanagement improves the outcomes of remediation and promotes the restoration of the ecosystems. Such findings indicate the possibilities of scalable, low-impact fungi-based remediation methodologies in the prevention of current and future challenges of hydrocarbon pollution by providing both quantitative and mechanistic data.

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Authors' Declaration

The authors declare that this manuscript is an original work, has not been published previously, and is not under consideration for publication elsewhere. All authors have made substantial contributions to the conception and design of the study, data collection, analysis and interpretation of the results, manuscript preparation, and approval of the final version. The authors take full responsibility for the accuracy and integrity of the work presented in this manuscript.

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Competing Interests

The authors declare that they have no known financial or non-financial competing interests, personal relationships, or conflicts of interest that could have appeared to influence the work reported in this manuscript.

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