MYCOREMEDIATION STRATEGIES FOR SOIL CONTAMINATION: AN UPDATED REVIEW OF *IN VITRO* APPROACHES, LIMITATIONS AND CHALLENGES FOR FIELD APPLICATION

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Abstract. This review provides a comprehensive overview and systematic synthesis of the literature on the application of mycoremediation for the treatment of contaminated soils. It highlights the taxonomic diversity of effective fungal species particularly Basidiomycetes (*Pleurotus ostreatus, Trametes versicolor*), and Ascomycetes (Aspergillus, Penicillium) and their key enzymatic mechanisms, primarily ligninolytic enzymes such as laccase (Lac), manganese peroxidase (MnP), lignin peroxidase (LiP), and cytochrome P450 monooxygenases (CYP), in the remediation of various pollutants including heavy metals, hydrocarbons (PAHs, PHs), and pesticides. These fungi effectively degrade PAHs, PHs, pesticides, and immobilize heavy metals through biosorption and bioaccumulation. Biostimulation strategies, through the addition of nutrient amendments (chitin, surfactants), as well as bioaugmentation with selected (indigenous or optimized) fungal strains, are significantly effective in increasing enzymatic activity and degradation rates (up to 70-98%), often outperforming phytoremediation approaches, as demonstrated in laboratory, pilot-scale, and field trials (LIFE MySOIL project, Niger Delta studies). However, field-scale implementation remains limited by environmental heterogeneity, inoculum viability, and a lack of long-term ecological impacts, and limited multi-omics integration. This analysis identifies critical research gaps in fungal-microbe interactions, enzymatic stability under field conditions, and standardized protocols, while confirming the promising potential of mycoremediation as a cost-effective (< €75/m^3) sustainable ecological strategy. Future research should prioritize *in situ* validation, genomic strain engineering, and integrated bioremediation strategies, while emphasizing adaptive approaches based on biological-environmental interactions to enable field-scale applications.

Keywords: *Basidiomycetes, Ascomycetes, ligninolytic enzymes, pollutants, biostimulation, bioaugmentation*

Introduction

Research into the application of mycoremediation in the treatment of contaminated soils has emerged as a major area of investigation, in response to the increasing prevalence of soil pollution due to industrial, agricultural and urban activities, which pose significant risks to ecosystems and human health (Pozdnyakova et al., 2022; Akpasi et al., 2023). Since the early 2000s, this field has expanded beyond laboratory studies on fungal degradation of specific pollutants to more integrated approaches using indigenous fungal communities and associated enzyme systems (Stella, 2020; Mathur and Gehlot, 2021). The practical value of mycoremediation lies in its cost-effectiveness, environmental compatibility and ability to remediate complex organic and inorganic contaminants, such as polycyclic aromatic hydrocarbons (PAHs), heavy metals, and pesticides (Ray et al., 2023). For example, petroleum hydrocarbons (PHs) are a major source of soil pollution worldwide, with degradation rates significantly enhanced by fungal enzymatic activity (Mahmud et al., 2022; Efenudu, 2024). The growing number of contaminated sites worldwide underlines the urgency of developing sustainable remediation technologies (Akpasi et al., 2023; Akram et al., 2023). The specific problem highlighted in this review is the insufficient understanding of the diversity, mechanisms and field applicability of fungi in the remediation of complex contaminated soils, particularly those with organic and inorganic co-contamination (Li et al., 2020; Hidalgo et al., 2023). Despite many studies demonstrating the ability of fungi to remediate PAHs, heavy metals and pesticides, there are still gaps in the optimization of fungal strains, the expression of pertinent enzymes and the influence of environmental factors on remediation efficiency (Akram et al., 2023; Pujiati et al., 2024; Crittenden et al., 2025). Controversies also remain over the relative efficacy of Basidiomycetes versus Ascomycetes, the role of indigenous versus introduced fungi and the transfer of laboratory results to field conditions (Germain et al., 2021; Akpasi et al., 2023; Crittenden et al., 2025). Failure to answer these questions could limit the operational implementation of mycoremediation, thereby prolonging the environmental and health risks (Geris et al., 2024). Conceptually, mycoremediation integrates fungal biodegradation, biosorption and biotransformation processes, mediated by extracellular enzymes such as laccases and peroxidases, which facilitate the degradation of pollutants and the immobilization of metals (Pozdnyakova et al., 2022; Ray et al., 2023). The interaction between fungal metabolic pathways and the physicochemical properties of the soil determines the results of remediation, establishing a strong link between fungal ecology and environmental biotechnology (Mathur and Gehlot, 2021; Maheshwari et al., 2024). This conceptual framework supports the research objective of understanding the specific capacities of fungal species and optimizing the conditions for effective soil decontamination. The aim of this systematic review is to evaluate current knowledge of fungal species, enzymatic mechanisms and environmental factors influencing mycoremediation of contaminated soils, including contributions from laboratory and field studies (Akpasi et al., 2023). This synthesis aims to fill knowledge gaps by consolidating diverse findings, in order to guide future research and practical applications in sustainable soil remediation (Stella, 2020; Ray et al., 2023). The methodology adopted is based on a comprehensive review of the peer-reviewed scientific literature on fungal remediation of organic and inorganic soil contaminants, using a thematic synthesis approach to organize the results according to fungal taxa, enzymatic activity and remediation results (Mathur and Gehlot, 2021; Verasoundarapandian et al., 2021; Singh et al., 2024). The following sections are organized around the diversity of fungi involved, the mechanisms of pollutant degradation, the factors influencing efficacy and the challenges associated with implementation in the field.

Objective of the study

This search focused exclusively on peer-reviewed publications, applying a time restriction to select the most recent articles. The objective is to synthesize current knowledge on the application of mycoremediation for the treatment of contaminated soils. Specifically, it aims to evaluate the effectiveness of fungal remediation techniques on a range of contaminants and identify gaps for future research. This review is particularly significant due to the substantial environmental and health risks posed by soil contamination from organic and inorganic pollutants, while mycoremediation presents a sustainable and cost-effective alternative to conventional remediation methods. By critically analyzing fungal species diversity, enzymatic mechanisms and biostimulation strategies, the report seeks to provide a comprehensive understanding of the potential and limitations of mycoremediation, thereby guiding the development of optimized, large-scale remediation technologies.

Description of the study

This review gives an overview of research on the application of mycoremediation in the treatment of contaminated soils, including a wide range of fungal species, contaminants, enzymatic mechanisms and remediation strategies. The studies reviewed cover fungal diversity ranging from Basidiomycetes and Ascomycetes to indigenous and metallotolerant strains, with a particular focus on the remediation of heavy metals, hydrocarbons, and pesticides. The methodologies used range from laboratory to pilot scale experiments, as well as field trials in various geographical regions, highlighting both fundamental enzymatic pathways and applied biostimulation or bioaugmentation techniques. This comparative analysis answers key questions about the role of fungi, enzymatic activity, environmental influences and the practical scale of application, providing valuable information for the future optimization of mycoremediation technologies.

Diversity of fungal species

Around twenty studies have reported a broad taxonomic range of fungi (*Table 1*), including Basidiomycetes, Ascomycetes, and indigenous metallotolerant strains, highlighting the diversity of fungal candidates for mycoremediation (Fallahi et al., 2023; Akram et al., 2023; Crittenden et al., 2025). Although traditional research has focused on Basidiomycetes, several studies emphasize the role of Ascomycetes in the degradation of PAHs, indicating their potential contribution under specific conditions (Champramary et al., 2023; Crittenden et al., 2025). Some work has concentrated on particular species such as *Pleurotus ostreatus*, *Trametes versicolor*, and *Ganoderma lucidum*, due to their efficient enzymatic systems (Efenudu, 2024). Among the diverse candidates, ligninolytic Basidiomycetes, particularly *Trametes* and *Pleurotus*, have consistently shown the highest efficiency in degrading persistent organic pollutants, especially in solid-state systems with lignocellulosic support or surfactant enhancement (Pozdnyakova et al., 2022). In contrast, filamentous Ascomycetes, including *Aspergillus*, *Penicillium*, and *Fusarium*, are most effective for heavy metal remediation, primarily via biosorption and bioaccumulation, especially under bioaugmentation or biostimulation strategies (Aslam et al., 2025).

Efficiency of contaminant degradation

Degradation efficiencies (*Table 1*) vary widely from study to study, with some fungal species achieving over 90% reduction of hydrocarbons, and pesticides, while the elimination of heavy metals is often reported in levels of biosorption or bioaccumulation capacity (Rathankumar et al., 2022; Akram et al., 2023). Degradation rates for PAHs range from a moderate efficiency of around 50% to almost complete degradation of over 90%, depending on fungal species and experimental conditions used (Efenudu, 2024; Zhang et al., 2024; Crittenden et al., 2025). Significant reductions in heavy metal pollution have been observed, often exceeding 50%, with certain fungal species demonstrating high tolerance and significant accumulation capacities (Mohamadhasani and Rahimi, 2022; Ray et al., 2023; Tagyan et al., 2023).

Enzymatic activity profiles

Enzymatic activity profiles (*Table 1*) show that ligninolytic enzymes, such as laccase, manganese peroxidase, and lignin peroxidase, are regularly identified as the main mediators of fungal degradation of organic pollutants (Mohamadhasani and Rahimi, 2022; Li et al., 2024; Crittenden et al., 2025). Moreover, antioxidant enzymes, notably superoxide dismutase and catalase, supporting fungal tolerance and their ability to detoxify heavy metals (Liu et al., 2023). Genomic and transcriptomic studies have highlighted families of genes encoding degradative enzymes, such as benzoate monooxygenases and cytochrome P450s, which support the potential of fungi in bioremediation (Park and Choi, 2020; Champramary et al., 2023).

Main physiological and biochemical mechanisms involved in mycoremediation

Mycoremediation relies on a combination of extracellular and intracellular physiological processes that enable the transformation, detoxification or immobilization of a wide range of organic and inorganic pollutants (Dinakarkumar et al., 2024). The first major process involves extracellular oxidative degradation, mainly mediated by ligninolytic enzymes such as laccases, manganese peroxidases, and lignin peroxidases. These enzymes catalyze non-specific oxidation reactions, generating free radicals able to cleave aromatic rings and destabilize complex and recalcitrant organic pollutants, including PAHs, PHs, pesticides, and dyes (Pozdnyakova, 2012; Dinakarkumar et al., 2024; Wang et al., 2024). The second key process is intracellular metabolic transformation, in which enzyme systems such as monooxygenases, dehydrogenases, and cytochrome P450 transferases catalyze hydroxylation, oxidation and conjugation reactions. These processes increase the solubility of pollutants and promote further metabolism, mineralization, or detoxification. Transporters such as ABC (ATP-binding cassette) and SLC (solute carrier) proteins, as well as endocytosis, play a key role in the capture and intracellular transport of degradation intermediates (Syed and Yadav, 2022; Luo et al., 2024). In the case of heavy metals, fungi use two complementary mechanisms: biosorption and bioaccumulation. Biosorption is passive, where fungal biomass fixes metal ions to the surface of the cell wall. Bioaccumulation is active and depends on metabolism, through which metals are absorbed and sequestered inside cells (Dinakarkumar et al., 2024). Overall, mycoremediation is mediated by the synergistic action of extracellular oxidation, intracellular metabolism, transport and sequestration mechanisms. This physiological polyvalence (*Figure 1*) explains the ability of fungi to target various contaminants and the variability observed between species and environmental conditions (Dinakarkumar et al., 2024; Wang et al., 2024).

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Key extracellular ligninolytic genes

White rot fungi and other saprophytic fungi host numerous laccase (lac/lcc) and class II peroxidase (mnp, lip, vp) genes in their genomes, with more than a dozen copies per family (Dao et al., 2021). RT-qPCR and transcriptomic analyses show that distinct subsets of these genes are selectively overexpressed upon exposure to hydrocarbons, PAHs, or lignin: rhizospheric strains of *Aspergillus* and *Trichoderma* upregulate the lig1-lig6 (LiP), mnp, and lcc genes in crude oil-based media, while *Trametes hirsuta* and *Dichomitus squalens* differentially induce specific mnp/vp genes and laccase isoenzymes in response to lignin-derived aromatic compounds (Asemoloye et al., 2018; Daly et al., 2019). Individual isoforms of *Rigidoporus* FMD21 laccase directly degrade dioxin, providing direct evidence that specific gene products are involved in xenobiotic degradation (Dao et al., 2021).

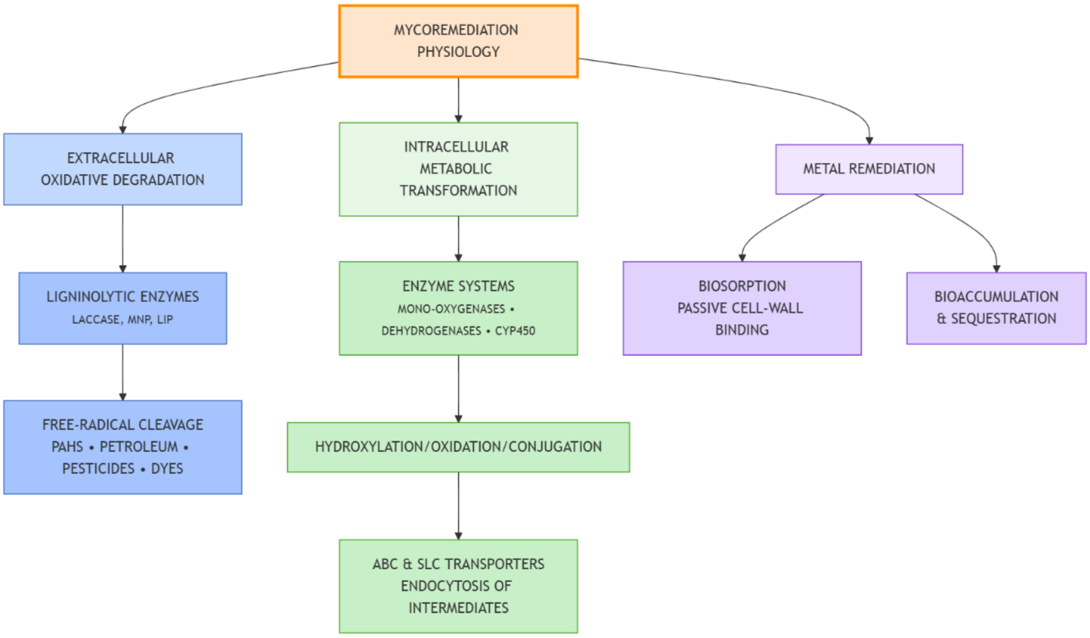

Figure 1. *Physiological and biochemical mechanisms involved in mycoremediation*

Intracellular oxidative and aromatic catabolic genes

Cytochrome P450 monooxygenase (CYP) genes are widely upregulated during growth on pure lignin or PAHs, triggering hydroxylation and O-demethylation. This response is observed in *Dentipellis* sp., *Trametes villosa*, and *Aspergillus sydowii* degrading PAHs under saline conditions (Park et al., 2019). Multi-omics analyses show the activation of oxidative and cleavage genes that drive lignin-derived aromatic compounds towards homogentisate, benzoate, and β-ketoadipate pathways (Peña et al., 2021).

Environmental regulation

Ligninolytic gene expression is strongly modulated by aromatic inducers, nutrient conditions and light, leading to selective upregulation of mnp/vp, cyp, gst, and related genes in several fungi (Lueangjaroenkit et al., 2020; Al-Zaban et al., 2021). Crude or aged oil spills similarly trigger high transcription of lig, mnp, lcc, and cat genes in rhizospheric

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fungi, in parallel with increased extracellular enzyme production and hydrocarbon tolerance (Al-Zaban et al., 2021). *Figure 2* maps the main fungal genes and enzymes involved in aromatic pollutant degradation and their integration into central catabolism.

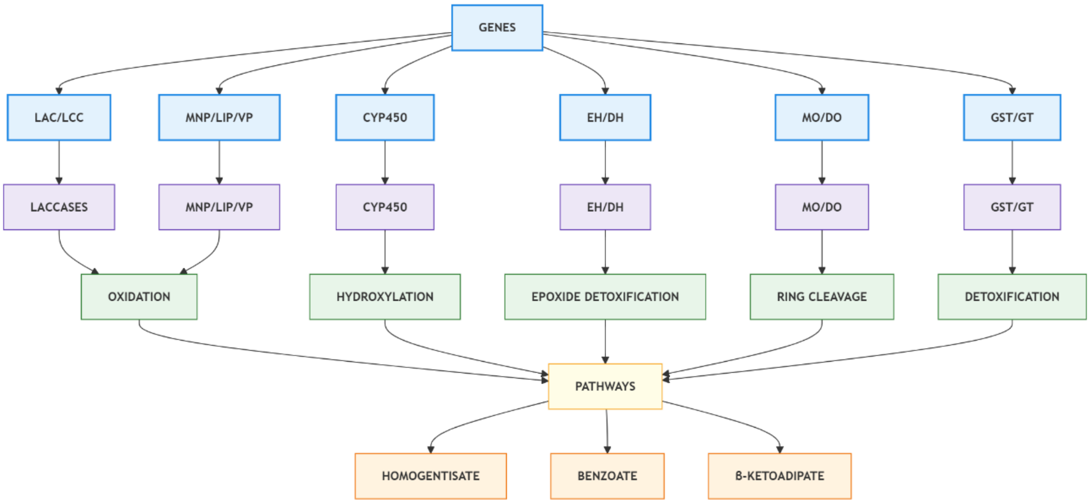

Figure 2. *Biodegradation-related fungal gene network.* ***LAC/LCC:*** *laccases/lignin-consuming copper-containing oxidases.* ***MNP/LIP/VP:*** *manganese peroxidase/lignin peroxidase/versatile peroxidase.* ***CYP450:*** *cytochrome P450 monooxygenases.* ***EH/DH:*** *epoxide hydrolase/dehydrogenase.* ***MO/DO:*** *monooxygenase/dioxygenase.* ***GST/GT:*** *glutathione S-transferase/glucosyltransferase*

Impact of biostimulation and bioaugmentation

The addition of nutrients, surfactants, and chitin-based substrates, as summarized in *Table 1*, improved fungal enzymatic activity and degradation rates in several studies (Rathankumar et al., 2022; Crittenden et al., 2025). Bioaugmentation with single fungal strains has often shown better performance than consortia or combined treatments, although synergistic interactions with bacteria have also been observed (Ule et al., 2021; Becarelli et al., 2021b). Finally, some research has highlighted the importance of optimizing substrates and inoculum size to boost the efficiency of bioremediation (Mirgeloybayat et al., 2023).

Dimensions and applicability

According to *Table 1*, pilot experiments and field trials in several studies illustrate the practical applicability of bioremediation in contaminated soils (Ule et al., 2021; Anoliefo et al., 2023). Field applications have often involved indigenous fungi adapted to local conditions, leading to the success of remediation operations (Anoliefo et al., 2023; Akram et al., 2023). In addition, reviews and genomic studies recommend scale-up and integrating omics technologies to optimize mycoremediation strategies (Akpasi et al., 2023; Champramary et al., 2023).

Critical analysis

The literature reviewed on the applications of mycoremediation in the treatment of contaminated soils reveals a significant interest in the diversity of fungal species, enzymatic mechanisms and biostimulation strategies. The strengths include the identification of various fungal taxa that can degrade a wide range of contaminants and the exploration of the enzymatic pathways associated with the transformation of pollutants. Another important point revealed that efficiency varies depending on environmental conditions. The high rates observed in laboratory or pilot studies are based on biostimulation (chitin, lignocellulose), but decrease in heterogeneous soils, limiting field-scale transfer. Enzymatic correlations have been established: ligninolytic enzymes (laccase, MnP, LiP) for organic compounds and antioxidant enzymes (SOD, CAT) for metal tolerance. Native strains outperform non-native strains and integrated approaches with other bioremediation methods optimize performance.

Strength points

Several studies have shown that various fungi, such as *Pleurotus ostreatus*, *Aspergillus*, and *Alternaria*, can efficiently remediate hydrocarbons, heavy metals, and pesticides in contaminated soils, due to their great adaptability and metabolic polyvalence (Cruz-Izquierdo et al., 2021; Fallahi et al., 2023; Ray et al., 2023). The identification of non-basidiomycete species able to degrade PAHs is expanding the range of fungi used for pollution control (Crittenden et al., 2025). Ligninolytic enzymes (laccase, MnP, LiP) are essential in the degradation of persistent pollutants, as demonstrated notably in *Trametes versicolor* and *Pleurotus ostreatus* (Park and Choi, 2020; Pozdnyakova et al., 2022; Champramary et al., 2023; Li et al., 2024). The addition of nutrient amendments (chitinous substrates and surfactants) and bioaugmentation with selected strains enhance enzymatic activity and improve soil decontamination (Ule et al., 2021; Rathankumar et al., 2022; Anoliefo et al., 2023; Crittenden et al., 2025). Mycoremediation often proves more effective than phytoremediation, particularly for heavy metals and hydrocarbons, and its combination with plants and biosurfactant microbes appears promising for soil remediation (Ahmad et al., 2023; Kalamulla et al., 2024). However, challenges remain: variability depending on the soil, poorly understanding of fungal ecology, viability under natural conditions, bioavailability of pollutants and complex environmental factors (Stella, 2020; Li et al., 2020; Ray et al., 2023; Hidalgo et al., 2023; Geris et al., 2024). Genomic and transcriptomic tools allow better selection and engineering of fungi to improve their remediation capacities (Park and Choi, 2020; Davolos et al., 2021; Champramary et al., 2023). Finally, mycoremediation also promotes the restoration of microbial communities, nutrient cycling and plant growth, contributing to overall ecological restoration (Tomer et al., 2021; Hidalgo et al., 2023; Kalamulla et al., 2024).

Weak points

Despite the diversity of fungi studied, research often focuses on a few strains, omitting the entire fungal community and its interactions with other soil micro-organisms. Most laboratory work limits understanding of fungal performance under complex natural conditions, while variability under different soils and contaminant mixtures is poorly studied, restricting the generalization of results (Stella, 2020; Hidalgo et al., 2023; Crittenden et al., 2025). Enzyme studies are mainly *in vitro* and do not always reflect enzyme expression or stability in the field, where enzyme regulation under environmental stress and mixed pollutants is still poorly understood (Li et al., 2024; Crittenden et al., 2025). The transfer of biostimulations from the laboratory to the field is difficult because of environmental variability, microbial dynamics, and the long-term ecological impacts of amendments are underexplored (Hidalgo et al., 2023; Crittenden et al., 2025). Comparative analyses often lack standardization and are based on short or laboratory studies, without considering costs, scale-up or ecological impacts, making integration with other bioremediation techniques insufficiently studied. The lack of large-scale studies and long-term monitoring is evident, as is the lack of analysis of the regulatory, economic and logistical obstacles to large-scale application, as well as the risks associated with the introduction of non-indigenous fungi (Stella, 2020; Geris et al., 2024). Finally, despite advances in genomics, practical applications remain limited due to regulatory and ecological challenges, as well as the complexity of fungal genomes (Davolos et al., 2021; Champramary et al., 2023). The real contribution of mycoremediation to sustainable soil restoration and its interactions with indigenous biota are still poorly explored, as is the impact of fungal metabolites or residues on soil ecology (Hidalgo et al., 2023).

Thematic analysis of the literature

Mycoremediation is emerging as an important and sustainable approach to the treatment of contaminated soils through the use of various fungal species able to degrade or immobilize organic and inorganic pollutants. The main research themes include characterization of fungal species and enzymatic mechanisms essential for pollutant degradation, evaluation of remediation efficiency for contaminants such as heavy metals, hydrocarbons, and pesticides and strategies such as biostimulation and bioaugmentation to enhance the remediation capacities of fungi (*Table 2*). Comparative analyses with other bioremediation techniques and the exploration of genomic and biotechnological advances optimize mycoremediation applications. The challenges posed by environmental factors, the complexity of pollutants and field deployment are also recurrently discussed, highlighting areas for future research (*Table 2*).

Chronological and bibliometric analysis of the literature

To refine the chronological perspective, we performed a bibliometric mini-study of the mycoremediation literature over the last two decades. Structured searches were conducted in the Web of Science Core Collection and Scopus using the following Boolean string: TS = ((mycoremediation OR "fungal remediation" OR "white-rot fungi") AND (bioremediation OR detoxification OR pollution OR contamination)). Document types were restricted to articles and reviews published in English, excluding duplicates and editorials. Three distinct phases can be identified (*Figure 3*). The period 2005–2009 was characterized by initial growth, with no more than 18 papers published per year. This was followed by a phase of accelerated expansion between 2010 and 2016, during which an average of 46 ± 8 papers were published annually. This period coincides with the first field-scale trials involving *Pleurotus* and *Phanerochaete* species. Finally, the 2017–2025 period represents a plateau with mild oscillations, averaging 71 ± 6 papers per year, corresponding to thematic maturation and a growing interest in nano-bio hybrids and microbiome-assisted mycoremediation.

Consensus and divergence between studies

The literature reviewed shows a wide consensus on the efficiency of various fungal species in the treatment of different contaminants, such as heavy metals, hydrocarbons, and pesticides (*Table 3*). Most studies highlight the key role of ligninolytic and other degradative enzymes in these processes, while biostimulation and bioaugmentation often improve remediation results. However, there are differences in the specificity of fungal species, degradation efficiency according to the type of contaminant and the transfer of laboratory results to field conditions. These differences can be explained by the variability of the experimental protocols, the types of pollutants studied, the environmental contexts and the methodologies employed (*Table 3*).

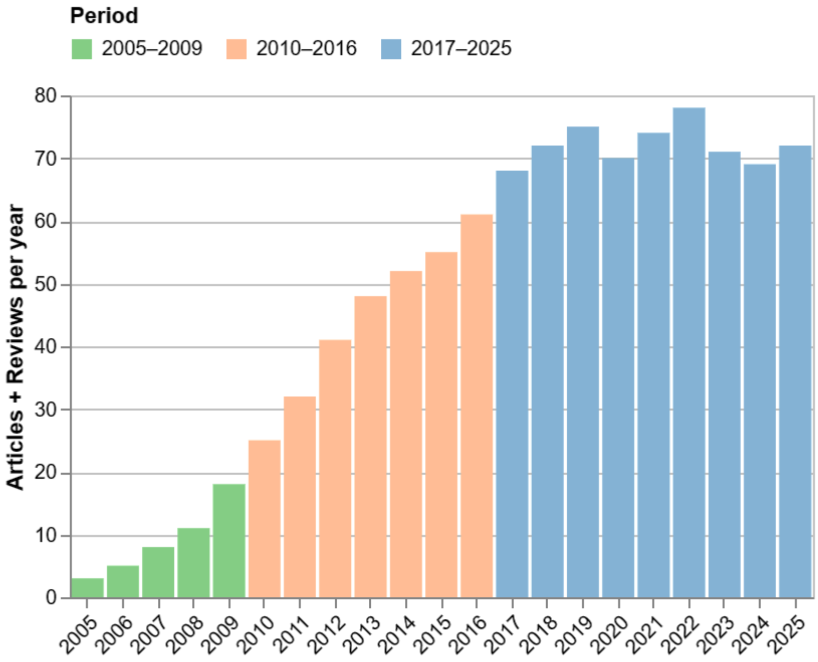

Figure 3. *Chronological and bibliometric analysis of mycoremediation research*

Theoretical and practical implications

Theoretical implications

The synthesis of results confirms the crucial role of fungal diversity, particularly Basidiomycetes and Ascomycetes, in the remediation of a wide range of soil contaminants, including PAHs, heavy metals, and pesticides. This supports existing theories on enzymatic versatility and the metabolic pathways involved in the transformation and mineralization of pollutants (Davolos et al., 2021; Shourie and Vijayalakshmi, 2022; Ray et al., 2023; Champramary et al., 2023). The evidence highlights enzymatic mechanisms, particularly ligninolytic enzymes (laccase, MnP, LiP) and cytochrome P450 monooxygenases, as being central to fungal degradation processes, confirming biochemical theories regarding the decomposition of pollutants by fungi (Jasu et al., 2021; Šrédlová et al., 2021; Champramary et al., 2023). Genomic and transcriptomic studies are providing new insights into the genetic basis of fungal biodegradation, revealing inducible genes and metabolic pathways that underlie the effectiveness of mycoremediation. They thus advance the theoretical understanding of fungal adaptation and functional gene expression in contaminated environments (Park and Choi, 2020; Davolos et al., 2021). The role of fungal bioaugmentation and biostimulation strategies, including the use of amendments such as chitin-based substrates, aligns with ecological theories on microbial community dynamics and synergistic interactions that promote pollutant degradation (Hidalgo et al., 2023; Crittenden et al., 2025). The influence of environmental factors such as pH, relative humidity, and soil physicochemical properties on fungal activity supports ecological theories regarding habitat suitability and microbial functional expression, highlighting the complexity of *in situ* mycoremediation processes (Li et al., 2020; Mohamadhasani and Rahimi, 2022).

Practical implications

Field and pilot-scale studies demonstrate that mycoremediation can be implemented as a cost-effective and environmentally compatible alternative to conventional soil remediation techniques, particularly for sites contaminated with heavy metals, PHs, and pesticides (Fu et al., 2021; Ray et al., 2023; Pujiati et al., 2024). In practice, the use of indigenous or site-adapted fungal strains consistently results in higher remediation efficiency and process stability, owing to their improved survival and metabolic performance under local soil and climatic conditions (Akram et al., 2023; Mirgeloybayat et al., 2023; Tatarin et al., 2024). Operational field strategies combining fungal bioaugmentation with biostimulation such as the addition of organic substrates, lignocellulosic residues, or biosurfactants have proven effective in enhancing contaminant removal by promoting fungal establishment and sustained enzyme production (Ahmad et al., 2023; Hidalgo et al., 2023). Solid-state and wood-assisted systems are particularly suitable for *in situ* soil remediation, as they provide physical support and nutrient sources that facilitate fungal persistence and activity in heterogeneous soil matrices (Pujiati et al., 2024). From a practical standpoint, field evidence indicates that mycoremediation performs best when integrated into multi-technology remediation frameworks, including phytoremediation and microbial consortia, rather than applied as a standalone approach.

Case studies and recent projects, including large-scale treatments, are being carried out by the LIFE MySOIL project, which has validated mycoremediation on a pilot and industrial scale at three European sites with contrasting contamination and climatic conditions (France, Italy, Spain), combining it with conventional processes and comparing it with them. The trials demonstrated operational feasibility, large-scale production of fungal inoculants, effectiveness on soils contaminated with hydrocarbons, (including long-chain hydrocarbons) and the transferability of the approach to other industrial pollutants. Economically, the treatment cost is less than €75 per m^3 of soil (less than €112.5 per tonne), compared to more than €100 per m^3 for conventional methods such as thermal desorption, representing a reduction of at least 25%. This competitiveness is based on reduced energy consumption, limited use of chemical reagents and the recovery of agricultural waste. Environmentally, the process reduces energy consumption by around 90% and significantly lowers greenhouse gas emissions (Herrero, 2025).

Oil-contaminated soils in the Niger Delta, Nigeria, show that *Pleurotus ostreatus*, combined with fermented palm wine and Tween 80, can achieve PH reduction rates of over 98% depending on soil texture, with maximum efficiency in loamy soils (Dickson, 2025). Earlier, Anoliefo et al. (2023) evaluated *Pleurotus tuberregium* for remediation in

Ogoniland, Nigeria, monitoring both fungal (*Mucor, Aspergillus, Penicillium*) and bacterial (*E. coli, Pseudomonas, Bacillus*) communities and observed progressive hydrocarbon and heavy metal degradation over four months via gas chromatography. Similarly, Ule et al. (2021) assessed *Mucor racemosus* and *Aspergillus niger* on crude oil-contaminated soils in Rivers State, Nigeria, using a 28.43 m^2 experimental area with a Randomized Block Design over 56 days, confirming effective hydrocarbon breakdown through weekly analyses. These findings confirm that mycoremediation is now an operationally mature technology that can be transferred on a large-scale.

Main limitations, gaps and future directions for research

Studies on mycoremediation have limitations, such as the lack of field experiments, the limited fungal diversity studied, insufficient analysis of environmental factors and long-term monitoring. To resolve these obstacles, future research should focus on the *in situ* validation of biostimulation strategies, the study of microbial interactions in complex contaminated soils, enzymatic stability in real-life conditions and the genomic engineering of fungal strains (*Tables 4 and 5*).

General overview and conclusion

This comprehensive review underscores the significant potential of mycoremediation as an environmentally friendly and economically viable strategy for remediating contaminated soils. Our analysis show that Basidiomycetes and Ascomycetes are particularly effective at degrading and immobilizing a wide range of pollutants through ligninolytic enzymes (laccases, MnP, and LiP) and P450 systems. Biostimulation and bioaugmentation strategies significantly enhance decontamination rates, confirming their potential to improve conventional remediation technologies. The targeted use of these fungal species and combined strategies demonstrates a tangible potential to reduce chemical inputs and energy consumption in large-scale applications.

However, large-scale effectiveness remains limited by environmental variability, inoculum survival, and the lack of long-term data. These findings highlight the need for long-term field trials, integration of omics approaches to optimize fungal strains, and the development of standardized protocols to enable practical and sustainable application of mycoremediation.

To address these challenges and maximize the potential of this technology, several recommendations can be formulated. Further efforts should focus on deepening fundamental knowledge of fungal systems, particularly through more in-depth studies of the diversity and ecology of indigenous fungal communities in contaminated soils, with special attention to complex microbial interactions. In parallel, genomics and transcriptomics approaches should be further integrated to elucidate specific degradation pathways, identify inducible genes, and optimize fungal strains through targeted genetic engineering. In addition, the stability and regulation of key enzymes, including laccases and peroxidases, should be characterized *in situ* under fluctuating field conditions.

Validation and optimization of field applications also represent essential priorities, requiring comprehensive, long-term, large-scale field studies with continuous monitoring to evaluate sustained effectiveness, ecological impacts, and residual contamination. Biostimulation strategies, such as chitin amendments and biosurfactants, as well as bioaugmentation using optimized inoculants, should be validated directly under field conditions while accounting for environmental heterogeneity. Furthermore, integrated approaches combining mycoremediation with other bioremediation techniques, including phytoremediation and bacterioremediation, should be developed to achieve more complete and resilient decontamination, particularly in cases of mixed contamination.

Finally, practical and regulatory aspects must be addressed to facilitate large-scale deployment. Application protocols should be standardized and optimized, from inoculation procedures to substrate management, in order to support technology transfer. Comprehensive economic assessments and life cycle analyses should be incorporated to demonstrate the cost-effectiveness of mycoremediation relative to conventional remediation methods. In parallel, appropriate, transparent, and science-based regulatory frameworks should be established to facilitate the adoption and large-scale deployment of fungal technologies. Collectively, these perspectives provide strong support for the practical potential of mycoremediation while emphasizing the importance of continued field validation, strain optimization, and protocol standardization to achieve sustainable large-scale applications.

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APPENDIX

Table 1. *Summary of studies on mycoremediation: fungal diversity, efficacy and biostimulation*

Studies	Diversity of fungal species	Efficiency of contaminant remediation	Enzymatic activity profiles	Impact of biostimulation and bioaugmentation	Dimensions and applicability
(Bharath and Gopalram, 2023)	*Pleurotus ostreatus* in municipal and oil soils	68-97.5% degradation of heavy metals and hydrocarbons	Laccase and other oxidative enzymes	Nutrient amalgamation in bioreactor enhanced degradation	Laboratory comparative with phytoremediation
(Crittenden et al., 2025)	132 isolates, mainly Ascomycota	Up to 58.3% PAH removal after biostimulation	Laccase and manganese peroxidase upregulated	Chitin-based amendments (grasshopper) increased enzyme activity and PAH removal	Laboratory isolation and biostimulation assays
(Fallahi et al., 2023)	265 isolates, 16 genera including *Alternaria*, *Fusarium*	>70% degradation of long-chain hydrocarbons in soil	Not detailed	Indigenous fungi applied directly to contaminated soil	Laboratory and soil inoculation experiments
(Giovannini et al., 2024)	101 taxa, 546 strains from military site	Several species utilized PAHs even with metal co-contamination	Functional traits screened, ligninolytic enzymes noted	PAHs enrichment microplate with Zn, Pb co-contamination	Laboratory microplate and functional screening
(Rathankumar et al., 2022)	*Trametes hirsuta* with wood-assisted fungal system	Up to 100% PAH degradation with surfactants	Enhanced enzyme production with rhamnolipids	Surfactant addition significantly improved degradation	Microcosm soil remediation study
(Anoliefo et al., 2023)	*Pleurotus tuberregium* and indigenous fungi	>95% reduction in aliphatic hydrocarbons, significant heavy metal reduction	Not detailed; microbial community monitored	Fungus amendment accelerated remediation compared to control	Field-scale remediation over 4 months
(Ule et al., 2021)	*Mucor* racemosus and *Aspergillus niger*	33% TPH reduction in 56 days	Hydrocarbon utilizing bacteria and fungi increased	Bioaugmentation with single fungi more effective than consortia	Field experimental plots with randomized design
(Cruz-Izquierdo et al., 2021)	*Aspergillus oryzae* and *Aspergillus flavipes*	235 mg/L phenanthrene degraded in 28 days	Ligninolytic enzyme activity (lignin peroxidase)	Degradation with and without additional carbon source	Laboratory culture and degradation assays
(Champramary et al., 2023)	10 armillarioid species and other Basidiomycota/Ascomycota	Genomic potential for aromatic degradation highlighted	benzoate monooxygenase and other enzymes	Transcriptome confirmed gene expression in wood-invading mycelia	Comparative genomics and transcriptomics
(Ray et al., 2023)	Filamentous fungi with heavy metal tolerance	High adsorption and accumulation of heavy metals	Multiple mechanisms: bioaccumulation, biosorption, biomineralization	Influenced by pH, temperature, metal concentration	Review of fungal bioremediation mechanisms
(Akram et al., 2023)	Indigenous metallotolerant fungi including *Aspergillus*, *Penicillium*	Efficient Pb and Cu removal from contaminated soil	Functional groups involved in metal uptake identified	Indigenous strains selected for *in situ* and *ex situ* use	Laboratory isolation and characterization
(Efenudu, 2024)	*Pleurotus ostreatus* strain	90% degradation of PAHs in spiked soil	Not detailed	Natural microbial community contributed to degradation	Laboratory soil spiking and incubation

Studies	Diversity of fungal species	Efficiency of contaminant remediation	Enzymatic activity profiles	Impact of biostimulation and bioaugmentation	Dimensions and applicability
(Li et al., 2024)	*Trametes versicolor*	69-88% BaP degradation; 16-56% TPH degradation	Laccase and intracellular protein correlated with degradation	Solid state fermentation most effective	Laboratory liquid and Solid state fermentation
(Mirgeloybayat et al., 2023)	*Auricularia auricularia-judae* strain	71% petroleum degradation after 45 days	Not specified, lignocellulosic waste used as substrate	Optimization of media and inoculum size	Laboratory Taguchi design optimization
(Becarelli et al., 2021a)	*Ciboria* sp. (Ascomycota)	78-99% TPH reduction in 90 days	Fungal metabolism accelerated bacterial specialist onset	Fungal inoculum dosage influenced degradation	Laboratory mesocosm with microbial community analysis
(Akpasi et al., 2023)	Various fungi including *Pleurotus dryinus*, *Trametes hirsuta*	91-98% degradation of pesticides and dyes	Ligninolytic enzymes and other degradative enzymes	Mycoremediation effective for metals and emerging pollutants	Review of fungal remediation efficacy
(Barman et al., 2023)	Macro- and micro-fungi including *Trichoderma*, *Aspergillus*	Biotransformation of PAHs, PCBs, heavy metals	Enzymes: laccase, manganese peroxidase, cytochrome P450	Mycorrhizal associations suport biodegradation	Review of fungal enzymatic roles in soil remediation
(Mohamadhasani and Rahimi, 2022)	*Pleurotus* sp.	Metal tolerance varies: growth inhibited by Ni at low concentration	Increased SOD and CAT activity up to 45 mg/L metals	Metal accumulation increased with concentration	Laboratory culture and enzymatic assays
(Pozdnyakova et al., 2022)	Basidiomycetes and Ascomycetes including *Pleurotus ostreatus*	Degradation of PAHs, dyes, oil without toxic metabolites	Ligninolytic enzymes and emulsifying compounds produced	Enzyme complex composition influenced degradation	Laboratory enzymatic and toxicity assays
(Tagyan et al., 2023)	*Curvularia*, *Fusarium*, *Penicillium*, *Trichoderma*	Fe(III) uptake capacities up to 72.46 mg/g	Tolerance index and adsorption isotherms analyzed	Optimal pH and temperature for Fe removal identified	Laboratory tolerance and adsorption studies

Table 2. *Thematic analysis of the literature*

Theme	Theme Description
Diversity and roles of fungi in soil contaminant degradation	Research has widely documented a wide diversity of fungal taxa, including Basidiomycetes and Ascomycetes, that can degrade various contaminants. Several studies highlight species such as *Pleurotus ostreatus*, species of the genus *Aspergillus*, and *Trichoderma*, which demonstrate their ability to decompose hydrocarbons, pesticides and heavy metals, using extracellular enzymatic activity and great metabolic polyvalence (Malik et al, 2021; Shourie and Vijayalakshmi, 2022; Bharath and Gopalram, 2023; Fallahi et al., 2023; Ray et al., 2023; Crittenden et al., 2025).
Enzymatic mechanisms and biochemical pathways in mycoremediation	Fungal degradation is mainly performed by ligninolytic enzymes such as laccase, MnP, and LiP, as well as intracellular enzymes such as cytochrome P450 monooxygenases. These enzymes catalyze the oxidative and reductive transformations of pollutants, facilitating their mineralisation or detoxification. Several studies have shown that it is possible to increase enzyme production using specific amendments and have demonstrated a correlation between enzyme activity and the efficiency of remediation of PAHs, pesticides, and heavy metals (Jasu et al., 2021; Park and Choi, 2020; Pozdnyakova et al., 2022; Champramary et al., 2023; Crittenden et al., 2025).
Mycoremediation of heavy metals in contaminated soils	Fungi have significant capacities for biosorption, bioaccumulation and biotransformation of heavy metals such as lead, copper, chromium, and arsenic. Filamentous fungi and macromycetes accumulate metals in their fruiting bodies, mobilize antioxidant enzymes to reduce metal-induced stress and modify the bioavailability of metals in the soil. Native and metal-tolerant fungal strains have been isolated and characterized for the remediation of heavily contaminated soils. In addition, several studies have explored the bioaugmentation and evaluation of fungal tolerance indices (Kumar and Dwivedi, 2021; Mohamadhasani and Rahimi, 2022; Bharath and Gopalram, 2023; Ray et al., 2023; Akram et al., 2023; Kamal et al., 2023; Tagyan et al., 2023; Patel et al., 2024).
Biostimulation and bioaugmentation strategies	Several research projects are being carried out to improve fungal pollution control using nutrient amendments, co-substrates (such as agricultural waste or chitin-based materials) or co-cultures with bacterial consortia. These interventions promote fungal growth, enzyme expression and pollutant transformation rates. Studies highlight the use of organic amendments such as compost, chicken feathers or sawdust as stimulants, with reported success in both microcosms and field conditions, although challenges remain in terms of scale-up (Becarelli et al., 2021b; Mirgeloybayat et al., 2023; Ahmad et al., 2023; Akpasi et al., 2023; Hidalgo et al., 2023).
Comparative effectiveness of mycoremediation versus other bioremediation techniques	Several studies have compared mycoremediation with phytoremediation and bacterial bioremediation, often concluding that fungi are more effective at degrading recalcitrant organic pollutants due to their robust enzymatic systems and high adaptability. However, combinations of fungi with plants (mycorrhizae) or bacteria often show synergistic effects that improve depollution performance. The limitations identified include slower degradation rates and a high dependence on environmental conditions, compared with some microbial approaches (Bharath and Gopalram, 2023; Hidalgo et al., 2023; Soni, 2024).
Genomic, transcriptomic and biotechnological advances in mycoremediation	Genomic sequencing and transcriptomic profiling are used to identify the genes involved in the degradation of pollutants, revealing the metabolic pathways and associated regulatory mechanisms. This molecular knowledge facilitates strain selection, enzyme engineering and the development of genetically improved fungi with enhanced degradation capacities. Omics approaches and biotechnological applications, including nanotechnology and genetic modification, are emerging areas for mycoremediation (Park and Choi, 2020; Davolos et al., 2021; Champramary et al., 2023).

Theme	Theme Description
Application of mycoremediation to specific contaminants: hydrocarbons, pesticides and emerging pollutants	The targeted remediation of PAHs, PHs, pesticides, and emerging contaminants such as pharmaceuticals has been demonstrated using a variety of fungal species. Studies characterize degradation kinetics, metabolite profiles and toxicity reduction, often focusing on agricultural and industrial pollution scenarios. The ability to degrade combined contaminants and co-pollutants is also being widely studied (Shankhwar and Paliwal, 2021; Landa-Faz et al., 2021; Rathankumar et al., 2022; Cruz-Izquierdo et al., 2021; Majid et al., 2024; Pujiati et al., 2024).
Challenges and limitations in field applications and scale-up	Despite promising results in the laboratory and on a pilot scale, a number of challenges persist, including the variability of the environment, the limited survivability of fungi or their restricted activity *in-situ*, the complexity of pollutant mixtures and the need for amendments adapted to each situation. Economic and technical obstacles are also holding back large-scale deployment, underlining the importance of integrated approaches and the need for further field validation (Stella, 2020; Dutta et al., 2023; Hidalgo et al., 2023; Akpasi et al., 2023; Geris et al., 2024).

Table 3. *Comparative summary of studies on mycoremediation: consensus, divergences and explanatory factors*

Comparison criteria	Consensus	Divergence	Possible explanation
Diversity of fungal species	The majority of studies agree on the implication of various fungal taxa, in particular Basidiomycetes and Ascomycetes, in mycoremediation. Species frequently reported to be effective degraders include *Pleurotus ostreatus*, *Trametes versicolor*, species of the genus *Aspergillus*, *Penicillium* and *Trichoderma* (Germain et al., 2021; Shourie and Vijayalakshmi, 2022; Fallahi et al., 2023; Chakraborty et al., 2023; Ray et al., 2023; Champramary et al., 2023; Crittenden et al., 2025). Indigenous fungi collected from contaminated sites are often preferred as they are better adapted to local conditions (Germain et al., 2021; Mirgeloybayat et al., 2023; Crittenden et al., 2025).	Some studies underline that ascomycete fungi are more efficient for the specific degradation of PAHs (Crittenden et al., 2025). Others highlight unique candidates such as *Auricularia auricularia-judae* and *Ciboria* sp. (Becarelli et al., 2021a; Mirgeloybayat et al., 2023). In addition, the efficacy of fungal consortia varies according to the type of pollutant and the site studied, highlighting a wide range of results (Becarelli et al., 2021b).	The differences observed can be explained in particular by local fungal biodiversity, the specificity of pollutants and the ecological adaptation of fungi. In addition, sampling methods and identification techniques, whether molecular or morphological, also influence the fungal diversity reported in the studies (Germain et al., 2023).
Degradation efficiency of contaminants	High rates of hydrocarbon and heavy metal elimination have been widely reported, with over 70-90% degradation of PAHs and PHs by species such as *Pleurotus, Trametes* and *Aspergillus* (Becarelli et al., 2021b; Rathankumar et al, 2022; Bharath and Gopalram, 2023; Fallahi et al., 2023; Li et al., 2024; Patel et al., 2024). Fungi have a high biosorption and bioaccumulation capacity for heavy metals such as lead, copper, chromium, and arsenic (Ray et al., 2023; Akram et al., 2023; Kamal et al., 2023; Patel et al., 2024). Biostimulation also considerably improves the efficiency of pollutant degradation (Rathankumar et al., 2022; Anoliefo et al., 2023; Crittenden et al., 2025).	Some studies report lower or variable degradation rates, particularly in the case of mixed or combined contamination (for example : PAHs and PHs), where the degradation of certain compounds is reduced. Bioaugmentation by fungi is sometimes less effective than co-composting or indigenous microbial activity (Becarelli et al., 2021b). In addition, tolerance to heavy metals varies considerably between fungal species (Mohamadhasani and Rahimi, 2022).	Differences in the complexity of the contaminants, the specificity of the fungal strains, the type of experimental protocol (laboratory/field), the concentration of the pollutants and the environmental conditions explain the variability in the degradation results.
Enzymatic activity profiles	It is widely admitted that ligninolytic enzymes, such as laccase, manganese peroxidase and lignin peroxidase, play a central role in the degradation, in particular, of PAHs and complex organic pollutants (Pozdnyakova et al., 2022;	Some studies report that the contribution of intracellular enzymes compared with extracellular enzymes varies according to the type of pollutant and the fungal species concerned. The increase in enzyme expression also depends on the	The variability results from the metabolic pathways specific to the different fungal species, the types of pollutants studied, the analytical methods used and the substrates used to stimulate enzyme production.

Comparison criteria	Consensus	Divergence	Possible explanation
	Champramary et al., 2023). Enzymatic activity is positively correlated with degradation efficiency, extracellular enzymes being essential for pollutant transformation (Rathankumar et al., 2022; Li et al., 2024). Oxidative and hydrolase enzymes are also widely involved in fungal degradation processes (Stella, 2020).	amendments used and the specific fungi, as shown by the increase in laccase activity in the presence of chitin-based substrates (Crittenden et al., 2025).	
Impact of biostimulation and bioaugmentation	The majority of studies agree that nutrient amendments or substrates (such as organic waste, chitin-based amendments, surfactants or sawdust) stimulate fungal enzymatic activity and improve remediation efficiency (Dubrovskaya et al., 2021; Rathankumar et al., 2022; Mirgeloybayat et al., 2023). Bioaugmentation with indigenous or specialised fungal strains accelerates degradation rates compared to natural attenuation (Anoliefo et al., 2023; Mirgeloybayat et al., 2023). In addition, biostimulation promotes both the degradation of PAHs and tolerance to heavy metals (Cruz et al., 2024; Crittenden et al., 2025).	Some studies show that the effects of cosubstrates can inhibit partner bacteria during fungal bioaugmentation, thereby reducing overall efficacy (Becarelli et al., 2021b). The evolution in the field and the transfer of the benefits of biostimulation observed in the laboratory remain uncertain (Anoliefo et al., 2023; Crittenden et al., 2025). In addition, the relative benefit of fungal consortia compared with single strains is sometimes the subject of debate (Ule et al., 2021).	The variations observed can be explained by the complexity of the pollutants, the types of amendments applied, the interactions within the microbial communities and the scale of the studies (laboratory or field). The success of bioaugmentation depends widely on the adequacy of the organisms introduced to the environmental conditions.
Scale and applicability	There is a consensus that mycoremediation is a promising and cost-effective approach at both laboratory and pilot scale, with several studies demonstrating *in situ* or *ex situ* soil remediation (Ule et al., 2021; Bharath and Gopalram, 2023; Mirgeloybayat et al., 2023; Anoliefo et al., 2023). Field trials show positive trends in terms of depollution, although they are often slower or less successful than the results obtained in the laboratory (Hidalgo et al., 2023; Geris et al., 2024). The use of indigenous fungi adapted to local soil conditions is preferable for field applications (Mirgeloybayat et al., 2023; Anoliefo et al., 2023).	Some studies highlight the challenges associated with scaling up mycoremediation, in particular variable performance of Basidiomycetes under field conditions (Hidalgo et al., 2023; Crittenden et al., 2025). The variability of environmental conditions and the sometimes incomplete elimination of pollutants, which remain below regulatory levels, complicate on-site efficacy (Hidalgo et al., 2023). Furthermore, the positive effects of biostimulation observed in the laboratory are not systematically found in field applications (Crittenden et al., 2025). The need to optimize protocols and strengthen validation through field trials is regularly highlighted in the literature (Geris et al., 2024).	The heterogeneity of the environment, the complexity of the pollutants, the adaptability of the fungi and the methodological differences between the laboratory and the field have an impact on application in real conditions. In addition, regulatory and practical limitations affect application in real-life conditions.

Table 4. *Main limitations in mycoremediation studies*

Limit	Description of the limit
Limitations in the field	Many studies are conducted on a laboratory or microcosm scale, which limits their external validity and practical applicability. The lack of field or *in situ* experiments limits understanding of the real efficacy and large-scale applicability of mycoremediation techniques.
Limited fungal diversity	Research often focuses on a limited number of fungal species, mainly Basidiomycetes or Ascomycetes, which limits the generalization of results. This focus may omit other fungi with remediation potential and limits overall ecological applicability.
Insufficient analysis of environmental factors	Many studies omit the influence of environmental parameters such as pH, temperature, soil type and humidity on the effectiveness of mycoremediation, which reduces the validity of the conclusions and limits the possibilities for optimizing conditions for field applications.
Lack of long-term monitoring	The short duration of the experiments limits understanding of the sustainability and long-term effects of mycoremediation. Without prolonged monitoring, the risk of contaminants reappearing or potential ecological impacts are not evaluated, which reduces confidence in the sustainability of remediation results.
Limited studies on several pollutants	Few studies treat the simultaneous remediation of mixed contaminants (for example : heavy metals associated with organic pollutants), a frequent situation in real soils. This gap limits the applicability of results to complex contaminated environments and reduces ecological validity.
Methodological constraints in assessing enzymatic activity	The variability and lack of standardization in the measurement of fungal enzymatic activities limits comparability between studies. This methodological limitation affects the validity of the links established between enzymatic activity and remediation efficacy.
Genetic and molecular mechanisms unexplored	A lack of genomic and transcriptomic data to clarify the degradation pathways of fungi, which limits understanding of the mechanisms and reduces optimization of the genetic or biotechnological processes of mycoremediation.
Limited geographical area	Many fungal isolates and studies from limited geographical regions may contribute to diversity and efficacy results. This geographical limitation reduces the overall applicability and ecological representativity of the results.
Limited integration with other bioremediation techniques	Few studies explore the synergistic effects of mycoremediation combined with phytoremediation, bioaugmentation or biostimulation, which limits our comprehension of integrated approaches that may improve the efficacy of remediation and its applicability in the field.
Incomplete evaluation of toxicity and by-products	Many studies do not thoroughly evaluate the toxicity of degradation by-products or the post-remediation ecological impacts, which are essential to ensure the environmental safety and regulatory acceptance of mycoremediation technologies.

Table 5. *Gaps and future directions for research*

Gap	Description	Future directions for research	Reasons	Research priority
Field validation of biostimulation strategies	Most biostimulation studies improving the enzymatic activity of fungi and degradation rates remain at laboratory scale, without validation at field scale.	Conducting controlled field trials to test chitin- and surfactant-based amendments to biostimulate fungi, evaluating long-term efficacy and ecological impacts under variable environmental conditions.	Successes achieved in the laboratory do not always mean effective application in the field, due to the complexity of environmental conditions. It is therefore crucial to carry out *in situ* validation to ensure the real performance of the methods (Hidalgo et al., 2023).	High
Interactions of fungal flora in soils with mixed contamination	Interactions between fungal species and between these fungi and indigenous microbial communities in soils contaminated by mixed pollutants, remain poorly studied.	Using metagenomic and metatranscriptomic approaches to characterize interactions between fungi and bacteria and the dynamics of microbial communities during the mycoremediation of contaminated soils.	Synergistic or antagonistic interactions can have a significant influence on remediation results, understanding them is essential for optimizing microbial consortia (Li et al., 2020).	High
Stability of enzymatic activity under field conditions	The stability, regulation and duration of activity of key fungal enzymes (for example : laccase, peroxidases) *in situ*, under the effect of environmental stress factors, are poorly characterized.	Looking for enzyme expression profiles and the persistence of enzyme activity in contaminated soils under real conditions over time, considering the influence of pH, temperature and pollutant mixtures.	Enzymatic degradation is crucial for mycoremediation; without stable enzymatic activity, the efficiency of remediation declines (Li et al., 2024; Crittenden et al., 2025).	High
Genomic and biotechnological integration for strain optimization	Despite advances in the genomics of fungal degradation pathways, practical applications of genetic engineering or strain optimization remain limited.	Developing genetically ameliorated fungal strains with overexpression of degradative enzymes and improved tolerance to pollutants, integrating multi-omics data to design targeted biocatalysts.	Genomic data provides a basis for improving fungal strains, but it is crucial to look for regulatory and ecological challenges in order to realize these benefits (Park et al., 2019; Davolos et al., 2021).	Average
Mycoremediation of heavy metals in complex soil matrices complex	Current studies focus on the remediation of a single heavy metal, while the efficacy and mechanisms of action of fungi in soils contaminated by several heavy metals and organic pollutants have yet to be fully explored.	Design experiments to evaluate fungal tolerance, accumulation and detoxification mechanisms in soils contaminated by several heavy metals and a mixture of organic and metallic pollutants, including evaluations of contaminant bioavailability.	Real contamination is complex, understanding fungal responses to mixed pollutants is essential to ensure applicability in the field (Li et al., 2020).	High
Comparison with other bioremediation methods	Few studies offer complete and standardized comparisons between mycoremediation and other bioremediation techniques such as phytoremediation or bacterial bioremediation.	Conducting comparative studies in the field and on a pilot scale using standardized indicators (degradation rates, costs and ecological impacts) to make a comparative evaluation of the efficacy of mycoremediation compared with alternative methods.	Comparative data is needed to justify the adoption of technologies and their integration into remediation approaches (Hidalgo et al., 2023).	Average

Gap	Description	Future directions for research	Reasons	Research priority
Optimization of fungal inoculum and substrate formulations	The influence of inoculum size, fungal strain combinations and substrate amendments on remediation efficiency is insufficiently optimized for field applications.	Systematically evaluate inoculum dosages, consortia versus isolated strains and lignocellulosic substrate types using factorial designs to optimize degradation and colonization.	Optimization can significantly increase degradation rates and process scale-up (Ule et al., 2021; Becarelli et al., 2021a; Mirgeloybayat et al., 2023).	Average
Long-term monitoring and evaluation of ecological impact	A lack of long-term studies monitoring soil health, the recovery of microbial communities and the possible side-effects of fungal remediation products.	Conducting longitudinal field studies to evaluate the physicochemical properties of soils, microbial diversity and plant growth after mycoremediation, in order to appreciate the sustainability and restoration of ecosystems.	Sustainable remediation requires the elimination of pollutants and the restoration of ecosystems (Barman et al., 2023).	High
Mycoremediation of emerging contaminants and pesticides	Research on fungal degradation of emerging pollutants (for example : pharmaceuticals, newly developed pesticides) and complex mixtures of pesticides is limited.	Studying fungal degradation pathways, the enzymatic mechanisms involved and the toxicity of metabolites produced during the degradation of emerging pollutants and pesticide cocktails in contaminated soils.	Emerging pollutants represent new challenges; fungi offer promising potential but require targeted studies to optimize their use (Kwatra and Abraham, 2023; Pujiati et al., 2024; Majeed et al., 2025).	High
Tolerance and toxicity levels of fungi	The inhibitory effects of some heavy metals such as nickel and pollutant concentrations on fungal growth and enzyme activity have not yet been fully quantified.	Define tolerance levels for the main fungal species under variable pollutant loads, develop strategies (for example : increasing antioxidant enzymes) to mitigate toxicity and maintain fungal activity.	Understanding tolerance limits is essential to avoid the failure of remediation operations due to toxicity (Mohamadhasani and Rahimi, 2022).	High