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Evaluation of indigenous fungi and their extracellular enzymatic profiles for effective mycoremediation of polluted soil environments

Khelil Bouziane Errahmani[1*], Abdesselem Si Mohammed[1], Ahmed Megharbi[2], Mustapha Djellouli[1] and Hayet Cherrad[3,4]
[1]Laboratory of Environment and Sustainable Development, Department of Biological Sciences, Faculty of Natural and Life Sciences, Ahmed Zabana University of Relizane, Algeria
[2]Laboratory of Environment and Sustainable Development, Department of Ecology and Environment, Faculty of Natural and Life Sciences, Ahmed Zabana University of Relizane, Algeria
[3]Department of Food Sciences, Faculty of Nature and Life Sciences, Abdelhamid Ibn Badis University of Mostaganem, Algeria
[4]Biochemistry, Molecular Biology and Environmental Toxicology Research Laboratory, Faculty of Medicine, Abdelhamid Ibn Badis University of Mostaganem, Algeria
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Abstract

Soil contamination by dyes, pharmaceuticals, and hydrocarbons poses a significant environmental challenge, requiring sustainable remediation strategies. In this study, four indigenous fungal strains—Psilocybe sp. (FK01), Schizophyllum sp. (FK02), Trichoderma sp. (FK03), and Aspergillus sp. (FK04) were isolated from diverse ecological niches for potential use in soil mycoremediation. Isolation was carried out using serial dilution and direct plating methods and the strains were subsequently cultured on Potato Dextrose Agar (PDA) medium supplemented with antibiotics. Pure cultures were obtained through repeated subculturing and monospore isolation. Fungal identification was based on macro-morphological characteristics, including colony morphology, pigmentation, growth patterns, and microscopic structures (hyphae, conidiophores, and phialides). Observations were performed using optical microscopy (400×–1000×) after lactophenol cotton blue staining. Enzymatic activities (catalase, protease, amylase, and manganese peroxidase) were evaluated on solid media with corresponding substrates, while laccase activity was measured spectrophotometrically using ABTS at 420 nm. Biodegradation efficiency was assessed under controlled conditions by monitoring pollutant absorbance over 20–60 min. Schizophyllum sp. (FK02) achieved the highest degradation (~47% at 40 min), followed by Trichoderma sp. (~36–45%), Psilocybe sp. (~10–25%), and Aspergillus sp. showing variable activity. Pre-colonized cereal-grain substrates improved fungal survival and activity compared to sawdust, maintaining viability for up to three weeks in soil. These results demonstrate, through multivariate analysis, that the selection of specific fungal strains and the optimization of inoculum delivery systems significantly enhance the effectiveness of in situ bioremediation of contaminated soils.

Keywords: Fungi, mycoremediation, polluted soils, enzymatic activity

Introduction

Soil pollution and its subsequent economic repercussions represent a critical global challenge, with recent estimates suggesting that over one-third of the world's arable land is currently degraded (Cachada *et al*., 2018; Hou *et al*., 2025). This environmental crisis is driven largely by anthropogenic activities, including the intensification of agriculture marked by the excessive application of pesticides and fertilizers and the expansion of industrial, mining, and transport sectors. These activities introduce a complex cocktail of pollutants into the pedosphere, ranging from heavy metals and hydrocarbons to emerging xenobiotics such as pharmaceuticals, microplastics, and synthetic dyes (Khan *et al*., 2024).

The persistence and bioaccumulation of pollutants like heavy metals, endocrine disruptors, and carcinogenic agents in the food chain, coupled with the rising threat of antibiotic resistance, pose a severe risk to global biodiversity and public health (Raimi *et al*., 2022; Fernández-Maquieira *et al.*, 2026).

***Email:** khelil.bouzianeerrahmani@univ-relizane.dz
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Soil degradation promotes the emergence of novel crop pests and significantly reduces agricultural yields, thereby jeopardizing global food security (Rodríguez-Eugenio *et al*., 2018). Consequently, there is an urgent need for effective, sustainable remediation technologies to restore fertile land and mitigate these multi-faceted risks.

Bioremediation offers a promising biological alternative to traditional physicochemical methods. While phytoremediation (utilizing plants like sunflowers) and bacterial degradation are widely studied (Wang *et al*., 2026; Haq *et al*., 2026). Mycoremediation (the use of fungi to break down or remove environmental pollutants) presents unique advantages compared to traditional remediation methods due to the distinctive biological and ecological properties of fungi (Dinakarkumar, 2024). Fungi possess expansive mycelial networks capable of penetrating contaminated soil matrices more thoroughly than other organisms, often making in situ application more cost-effective and comprehensive (Bosco and Mollea, 2019). Mycoremediation works through two main mechanisms. First, fungi secrete enzymes that break down complex pollutants into less harmful or harmless substances. Second, their mycelial networks adsorb and immobilize contaminants—such as heavy metals—through processes like biosorption and chelation, reducing their mobility and toxicity (Purnomo *et al*., 2013; Akpasi *et al*., 2023). Despite advances in microbial and enzymatic bioremediation, integrated approaches that combine strain isolation, enzymatic characterization, and inoculum optimization with quantitative degradation analyses across diverse pollutants remain limited. Additionally, the potential of emerging fungi such as *Psilocybe* spp., alongside established taxa like white-rot fungi (WRF) and *Trichoderma* spp., is still insufficiently explored.

The present study aims to develop a comprehensive framework for the mycoremediation of complex soil pollutants by following a four-stage experimental approach. First, we identify the biological agents through the isolation and taxonomic characterization of four indigenous fungal strains (*FK01–FK04*). Second, we evaluate the enzymatic secretome by screening for key extracellular enzymes such as laccase, manganese peroxidase, and catalase. Thirdly, the pollutant degradation capacity of these enzymes is assessed by analyzing the degradation rates of representative model pollutants, including the dye (methylene blue), the pharmaceutical compound (ciprofloxacin), and the steroid compound (cholesterol). Finally, we transition to the 'Execution' by optimizing a cereal-based carrier system for the delivery of a fungal consortium, assessing its stability and viability for *in situ* application. By bridging the gap between molecular enzymatic potential and practical soil inoculation, this work offers a targeted and scalable strategy for the restoration of contaminated terrestrial ecosystems.

Finally, to explore the relationships between fungal strains and their functional characteristics, multivariate statistical approaches are essential when dealing with complex datasets combining multiple enzymatic activities, degradation capacities, and growth parameters. In this context, Correspondence Factor Analysis (CFA) represents an appropriate and powerful tool for the analysis of contingency tables composed of semi-quantitative data. CFA enables the simultaneous examination of fungal strains and functional variables within a reduced factorial space, facilitating the identification of associations, functional profiles, and patterns of similarity among strains. This approach provides an integrative and ecologically meaningful framework to characterize fungal functional diversity and to highlight strains with potential applications in environmental biotechnology and bioremediation. It is further hypothesized that the selected enzymes will exhibit pollutant-specific degradation efficiencies influenced by chemical structure, physicochemical properties, and ecological origin, with more complex or hydrophobic compounds such as cholesterol showing slower degradation and enzymes derived from distinct ecological niches displaying unique degradation profiles.

Materials and Methods

Isolation and identification of fungal strains

Sampling sites and material collection

Samples were collected from four distinct ecological environments in Algeria during the rainy and winter season of 2024, periods optimal for fungal growth and carpophores development (Koskinen, 2023; Seddaiu *et al*., 2026):

- Djebel Afroun forest: Colonized wood fragments were gathered from decaying wood (36°25’55’’N, 2°34’39’’E).
- Ain Defla orchard: Rhizospheric soil was collected at a depth of 10 cm from a living fig tree (36°13’22’’N, 1°40’07’’E).
- Ain Defla municipal site: Hydrocarbon-polluted soil was sampled at a depth of 10 cm (36°13’24’’N, 1°40’07’’E).
- Baborforest lawn: Macroscopic fruiting bodies were surveyed and collected (36°12'28"N, 1°51'00"E).

All soil samples were stored in sterile containers to prevent external contamination.

Isolation procedures

Fungal isolation was performed using Potato Dextrose Agar (PDA) as the primary culture medium, supplemented with antibiotics to suppress bacterial growth, and incubated at 28°C for 7 days (Rapilly, 1968).

The procedures for fungal isolation and inoculation are detailed in Table 1. Primary isolation of fungi was carried out on PDA, a general-purpose medium widely used for the cultivation of filamentous fungi. The medium was prepared according to standard protocols, sterilized by autoclaving at 121 °C for 15–20 min, and poured into sterile Petri dishes. Samples were inoculated onto the medium using direct plating or dilution techniques and incubated at 25 ± 2 °C for 3–7 days. Emerging fungal colonies were regularly monitored for growth and morphological characteristics.

Purification and identification

Fungal isolates were purified by successive subculturing of well-isolated colonies onto fresh PDA plates. Colonies displaying distinct morphological characteristics were monitored via binocular microscopy or low-magnification light microscopy to ensure the absence of contaminating microflora and minimize contamination. Monospore isolation techniques were applied to obtain axenic cultures derived from a single propagule, ensuring both genetic uniformity and culture purity. Purified isolates were maintained on PDA slants at 4 °C for short-term preservation.

Fungal identification was conducted based on a combination of macroscopic and microscopic characteristics. Macroscopic evaluation included colony morphology on PDA, such as surface and reverse coloration, texture (cottony, velvety, or granular), topography, growth rate, margin configuration, pigment production, and the presence of exudates or distinctive odors. Observations were recorded after incubation under standardized conditions (Watanabe, 2010; Tibpromma *et al*., 2018).

Microscopic characterization was performed using light microscopy following staining with lactophenol cotton blue. Mycelial fragments were mounted on glass slides and examined at magnifications of 400× to 1000×. Diagnostic features included hyphal structure and septation, as well as the morphology of reproductive structures such as conidiophores, phialides, conidia, and sporangia, when present. Particular emphasis was placed on spore morphology (shape, size, and arrangement), conidial ontogeny, and the organization of spore-bearing structures (Jayasiri *et al*., 2015).

Enzymatic activity profiling

The metabolic potential of the isolates was thoroughly evaluated using a combination of solid and liquid culture media. This dual approach enabled a more comprehensive assessment of their growth characteristics, substrate utilization, and overall biochemical activity under different environmental conditions.

Qualitative screening on solid media

The isolates were screened for their ability to produce key enzymes (amylase, protease, catalase, laccase, and manganese peroxidase) involved in organic matter degradation and xenobiotic transformation. For all solid media tests, enzymatic production was confirmed by the formation of a distinct halo around the fungal colony following the addition of specific revealing agents. Negative controls consisted of uninoculated medium.

Protease activity: Assayed using a medium containing 5 g L^{-1} peptone, 10 g L^{-1} skimmed milk, and 15 g L^{-1} agar (pH 7.0). Proteolysis was indicated by a clear zone resulting from casein degradation (Kasana *et al*., 2011).

Amylase activity: Evaluated on a starch-agar medium (10 g L^{-1} starch, 10 g L^{-1} peptone, 15 g L^{-1} agar; pH 7.0). Starch hydrolysis was revealed through the addition of Lugol's iodine solution (Roth *et al*., 2019).

Table 1: Methods for fungal isolation and inoculation across diverse substrates

Sample type	Preparation & inoculation method	Antibiotic supplementation
Wood Fragments	Cut into 1cm × 2cm pieces, surface-disinfected with alcohol, and sliced longitudinally to expose endophytic fungi	Gentamycin & Ampicillin
Polluted soil and rhizospheric soil	10 g of soil diluted in 10 mL of sterile distilled water; inoculated via decimal dilution series	Streptomycin & Chloramphenicol (20 mg/100 mL)
Fruiting bodies	Carpophores were sectioned into 0.5cm ×0.5cm fragments and placed directly onto the agar surface	Streptomycin & Chloramphenicol

Manganese peroxidase (MnP) activity: Determined using a basal medium (10 g L^{-1} glucose, 10 g L^{-1} peptone, 0.1 g L^{-1} $MnCl_2$, 15 g L^{-1} agar) buffered to pH 6.0. Post-autoclaving, the medium was supplemented with 0.1 mM 2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and 10 mM H_2O_2 (Xu *et al*., 2017).

Catalase activity: Assessed by applying a drop of 3% H_2O_2 directly onto the fungal colony. The immediate evolution of oxygen bubbles indicated positive catalase production.

Laccase production and quantitative assay

To quantify laccase activity, isolates were cultured in a liquid medium (10 g L^{-1} glucose, 5 g L^{-1} peptone; pH 5.5). Erlenmeyer flasks were inoculated with mycelial plugs from active PDA cultures and incubated under agitation for seven days at 28°C. The fermented broth was harvested and centrifuged at 10,000 rpm for 15 minutes. The resulting cell-free supernatant, containing the extracellular metabolites, was utilized for the enzymatic assay.

Laccase activity was determined by monitoring the oxidation of ABTS). The reaction mixture contained 900 µL of 0.1 M acetate buffer, 50 µL of 1 mM ABTS, and 50 µL of the enzyme supernatant. Absorbance was measured at 420 nm every 30 seconds for 3 minutes (Galhaup and Haltrich, 2001). The calculation of the enzymatic activity is done in (U mL^{-1}) according to the following formula:

$$\text{Activity (U ml}^{-1}) = (\Delta A420\_min \times V\_total)/ (\varepsilon \times d \times V\_enzyme)$$

Where ΔA420_min is the absorbance variation per minute; V_totalis equal to 1 mL; ε is the molar extinction coefficient of ABTS (ε = 36000 $M^{-1}.cm^{-1}$); d is equal to 1 cm and V_enzyme is the supernatant volume.

Biodegradation of model pollutants by fungal enzyme extracts

To evaluate the catalytic efficiency of the isolated fungal secretomes, three model pollutants were selected representing diverse chemical classes: Methylene Blue (synthetic dye), Ciprofloxacin (pharmaceutical/antibiotic), and Cholesterol (polycyclic compound acting as a structural proxy for Polycyclic Aromatic Hydrocarbons (PAHs) and Endocrine disruptors). Reactions were monitored over a 60–120 minute period using a spectrophotometer.

Cholesterol degradation assay

The biodegradation of cholesterol was monitored through a coupled enzymatic reaction. The reaction mixture contained 3 mL of a 2 g L^{-1} cholesterol standard solution, 1 mL of culture supernatant, 1 mL of acetate buffer, and 1 mL of ABTS. In this system, the enzymatic breakdown of cholesterol generates hydrogen peroxide (H_2O_2), which subsequently drives the oxidation of ABTS. The resulting chromogenic change was measured spectrophotometrically at 500 nm in 20-minute intervals (Abdelwahed *et al*., 2021).

Methylene blue degradation

The degradation potential was assessed using a reaction mixture consisting of 1 mL of 50 µM methylene blue, 1 mL of phosphate buffer, 0.5 mL of 0.5 mM H_2O_2, and 0.5 mL of fungal supernatant. The reduction in absorbance, corresponding to the cleavage of the dye's chromophore, was recorded at 664 nm every 20 minutes (Almaz and Agircelik, 2023).

Ciprofloxacin degradation

The degradation of the antibiotic ciprofloxacin was evaluated by adding 0.5 mL of a 10 g L^{-1} ciprofloxacin stock solution and 0.5 mL of culture supernatant to 9 mL of phosphate buffer (pH 5.0). The reaction kinetics were monitored by measuring the decrease in absorbance at 276 nm (the characteristic peak for the quinolone ring) at 20-minute intervals (Singh *et al*., 2017).

Preparation and application of mycoinocula for soil bioremediation

To transition from lab to *in situ* application, mycoinocula were developed using two different carrier substrates. This section is critical. It demonstrates the importance of the carrier substrate and the pre-colonization phase for successful field implementation.

Carrier substrate preparation and Inoculation

Cereal grains and commercial sawdust were utilized as a solid support and nutrient carrier for the fungal isolates. The substrates were initially washed and subjected to a controlled humidification phase to achieve optimal moisture content for fungal proliferation. To ensure the eradication of endogenous microflora and prevent competition, the substrates were sterilized by autoclaving at 121°C for 20 minutes.

Under aseptic conditions, the sterile grains were inoculated with the fungal isolates and incubated at 28°C for 15 days. This incubation period allowed for the complete colonization of the substrate, resulting in a robust, uniform mycoinoculum.

In situ bioremediation experimental design

To evaluate the effectiveness of the mycoinocula in a real-world scenario, the fully colonized substrates were homogeneously incorporated into soil samples artificially contaminated with hydrocarbons. The evolution of the soil quality was monitored through periodic sampling to assess the degradation rate of the contaminants and the survival of the fungal inocula (Gupta *et al*, 2017).

Statistical analysis

To investigate the relationships between fungal isolates (FK01–FK04) and their functional profiles (enzymatic activities, substrate utilization, and growth rate); Correspondence Factor Analysis (CFA) was performed. CFA is a multivariate statistical method used to explore associations within contingency tables by simultaneously projecting rows (isolates) and columns (variables) into a reduced-dimensional factorial space. This approach enables

Table 2: Origin, place and year of isolation and taxonomy of the isolated strains

Isolates	Origin isolation site	Year of isolation	Taxonomic affiliation
FK01	Lawn Babor forest – Ain Defla 36°12’28’’N1°51’00’’E	2024	*Psilocybe* sp.
FK02	Tree trunk Djebel Afroun forest 36°25’55’’N2°34’39’’E	2024	*Schizophyllum*sp.
FK03	Rhizosphere orchard – Ain Defla36°13’22’’N1°40’07’’E	2025	*Trichoderma* sp.
FK04	Polluted soil municipal site – AinDefla 36°13’24’’N1°40’07’’E	2024	*Aspergillus* sp.

Table 3: Summary table of enzymatic activities of isolates on solid media

	Amylase	Protease	Mn POD	Catalase
FK01	+	+++	+	+++
FK02	++	+++	+++	+++
FK03	+++	++	+	+++
FK04	+++	+	+++	+++

Table 4: Extracellular laccase activity of fungal isolates in liquid culture

Strains	FK01	FK02	FK03	FK04
Laccase enzyme activity in U L^{-1}	1.1	4.5	3.6	5.2

Table 5: Contingency table (4 fungal strains × 12 variables)

Strain	Amylase production	Protease production	Mn-peroxidase production	Catalase production	Laccase production	Methylene blue degradation	Cholesterol degradation	Ciprofloxacin degradation	Growth rate	Growth on liquid medium	Growth on cereals	Growth on sawdust
FK01	1	3	1	3	1	1	3	1	1	1	1	1
FK02	2	3	3	3	3	3	1	2	3	3	2	2
FK03	3	2	1	3	2	3	2	3	3	3	2	2
FK04	3	1	3	3	3	2	1	3	3	3	3	3

intuitive visualization of similarities and differences among samples and their associated traits.

In this study, CFA was applied to Table 5, which consists of four fungal strains evaluated across twelve biochemical and growth variables. The data were structured on an ordinal scale ranging from 1 (low) to 3 (high), allowing comparative analysis of functional performance across strains.

Methodology

The analysis was conducted using a reproducible computational workflow based on Python and several specialized libraries for data handling, numerical computation, and visualization. Correspondence analysis was implemented using the Prince library, which provides efficient algorithms for multivariate analysis.

Implementation

The CFA model was initialized and fitted to the dataset as follows:

```
import prince
# Initialize Correspondence Analysis model
ca = prince.CA(n_components=2, n_iter=10, engine='sklearn')
# Fit the model to the contingency table
ca = ca.fit(df)  # df = contingency DataFrame
# Extract coordinates
row_coords = ca.row_coordinates(df)    # coordinates of fungal strains
col_coords = ca.column_coordinates(df) # coordinates of variables
```

The first two dimensions (F1 and F2) were retained, as they captured the majority of total inertia, allowing a reliable two-dimensional representation of the data structure.

Results and Discussions

Isolation and Identification of fungal strains

Following seven days of incubation, the isolates obtained from wood and carpophore fragments (FK01 and FK02), and the cultures were uniform, presenting white, diffuse mycelia. Their identification was established through a combination of the phenotypic macroscopic features of the original carpophores and the presence of hyaline, septate hyphae—a hallmark of Basidiomycetes. Based on these morphological characteristics, FK01 was assigned to the genus *Psilocybe* sp. and FK02 to *Schizophyllum* sp.

For the soil-derived samples yielded two distinct fungal morphotypes. Isolate FK03 exhibited fast-growing colonies with a cottony to floccose texture that later became more compact. The colony surface appeared white initially, turning green due to abundant conidial production, while the reverse remained pale. Microscopic examination revealed highly branched conidiophores with flask-shaped phialides arranged in clusters. The conidia were small, smooth, and oval, forming compact clusters at the tips of the phialides, which is characteristic of the genus *Trichoderma*.

Isolate FK04 showed rapid growth on PDA, forming dense colonies with a powdery to granular texture. Colony coloration ranged from green to dark green, with a pale to yellowish reverse. Microscopic examination revealed septate hyphae and characteristic conidiophores terminating in a vesicle bearing phialides. Chains of spherical conidia radiating from the vesicle formed a typical conidial head structure, which is diagnostic of the genus *Aspergillus*.

The origin, year, location of the isolation site, and taxonomic affiliation of the isolates are listed in Table 2.

Enzymatic activity profiling

Extracellular enzymatic profiling on solid media

The metabolic potential of the four isolates was evaluated through qualitative screening for four key enzymes. These activities are critical for the survival of the fungi in contaminated soils and their ability to degrade complex organic pollutants.

Amylolytic activity: Amylase production was confirmed by the formation of a distinct transparent halo around colonies of strains FK01 (*Psilocybe* sp.), FK02 (*Schizophyllum* sp.), and FK03 (*Trichoderma* sp.) following the addition of Lugol's iodine. This clearing zone indicates the secretion of endoamylases capable of complete starch hydrolysis. Conversely, strain FK04 (*Aspergillus* sp.) exhibited a significantly smaller degradation zone.

While the genus *Aspergillus* is a frequent industrial source of amylases, certain environmental strains, such as *A. flavus* and *A. fumigatus*, may exhibit negligible or unidentified amylase activity depending on specific inducing factors in their niche.

Proteolytic activity: Positive protease activity, evidenced by clear halos of casein hydrolysis, was observed for isolates FK02, FK03, and FK04:

- Isolate FK04 (*Aspergillus* sp.) demonstrated robust activity, consistent with its known ability to produce acidic, neutral, and alkaline proteases for broad substrate adaptation (Sandhya *et al*., 2005).
- Isolate FK03 (*Trichoderma* sp.) also showed significant proteolysis, which likely facilitates its mycoparasitic nature and organic matter decomposition.

- Isolate FK02 (*Schizophyllum* sp.) displayed versatility in nutrient exploitation, as previously noted by (Tovar-Herrera *et al*., 2018).
- In contrast, FK01 (*Psilocybe* sp.) lacked detectable protease activity. This enzymatic specialization suggests a genome primarily oriented toward the degradation of cellulose, hemicellulose, and lignin rather than proteins, a common trait in specialized basidiomycetes (Himmel *et al*., 2007).

Catalase activity: All four isolates exhibited vigorous effervescence upon the addition of hydrogen peroxide (H_2O_2), confirming the production of catalase. This enzyme is a critical defense mechanism against oxidative stress. In FK03 and FK04, catalase works in tandem with peroxidases and laccases to facilitate the degradation of toxic substances. For the basidiomycetes FK01 and FK02, catalase activity is essential for protecting mycelial integrity against reactive oxygen species (ROS) generated during the breakdown of complex recalcitrant pollutants (Pepe *et al*., 2025; Keller *et al*., 2025).

Manganese peroxidase (MnP) activity: The presence of Manganese peroxidase, a key lignin-modifying enzyme (LME), was identified by the formation of a dark precipitate in cultures of FK02 (*Schizophyllum* sp.) and FK04 (*Aspergillus* sp.). FK02's activity confirms its role as a primary lignin decomposer. The positive result for FK04 highlights the adaptability of certain *Aspergillus* strains to lignin-rich substrates (De Vries and Visser, 2001). Notably, FK01 and FK03 did not produce detectable levels of MnP under the tested conditions. This suggests that these strains may utilize alternative oxidative pathways (such as laccases or different peroxidases) for the degradation of aromatic compounds.

The results of the activity of the enzymes (Amylase, Protease, Mn POD and Catalase) investigated in the four strains are summarized in Table 3.

Quantitative enzymatic assay in liquid medium (Laccase Activity)

The quantitative data highlight distinct laccase production profiles among the four fungal isolates, reflecting both their ecological roles and evolutionary adaptations. Isolates FK04 (*Aspergillus* sp.) and FK02 (*Schizophyllum* sp.) demonstrated the highest laccase activities, reaching 5.2 U mL^{-1} and 4.5 U mL^{-1}, respectively, which is consistent with previous findings. Baldrian reported that ligninolytic fungi, particularly basidiomycetes such as *Schizophyllum*, are efficient laccase producers involved in lignin depolymerization (Baldrian, 2006). Similarly, Piscitelli *et al*. showed that *Aspergillus* species are capable of producing extracellular laccases with significant oxidative potential under suitable conditions (Piscitelli *et al*., 2011).

Isolate FK03 (*Trichoderma* sp.) exhibited intermediate laccase activity (3.6 U mL^{-1}), supporting observations by who described *Trichoderma* spp. as primarily cellulolytic organisms with auxiliary ligninolytic capabilities. In addition, demonstrated that certain *Trichoderma* strains can produce laccases to facilitate lignin modification and improve access to structural polysaccharides.

In contrast, FK01 (*Psilocybe* sp.) showed minimal laccase activity (1.1 U mL^{-1}), suggesting that alternative enzymatic systems may dominate lignin degradation in this isolate. As highlighted by Janusz *et al*. (2017), many basidiomycetes preferentially utilize manganese peroxidases and versatile peroxidases depending on environmental conditions and substrate composition. Furthermore, Sánchez emphasized that the expression of lignin-modifying enzymes is highly dependent on physicochemical parameters such as pH, temperature, and nutrient availability, which may explain the low laccase activity observed (Sánchez, 2009).

Overall, these findings align with established literature indicating that laccase production varies significantly among fungal taxa and is closely linked to their ecological function and enzymatic specialization, reinforcing the potential of *Aspergillus* and *Schizophyllum* species in mycoremediation applications.

Biodegradation of model pollutants by fungal enzyme extracts

The catalytic efficiency of crude enzymatic extracts from four fungal strains was assessed using three structurally distinct model pollutants: Methylene Blue (dye), Ciprofloxacin (pharmaceutical), and Cholesterol (polycyclic aromatic hydrocarbon analog). The degradation profiles followed pseudo-first-order Michaelis–Menten kinetics, indicating a strong affinity between fungal extracellular enzymes and the target substrates (Figure 1). This behavior is typical of ligninolytic enzymes, particularly laccases, which are known for their broad substrate specificity and high catalytic efficiency toward aromatic compounds. Baldrian and Piscitelli reported similar kinetic patterns, highlighting the adaptability of fungal laccases to diverse xenobiotics (Baldrian, 2006; Piscitelli *et al*., 2011).

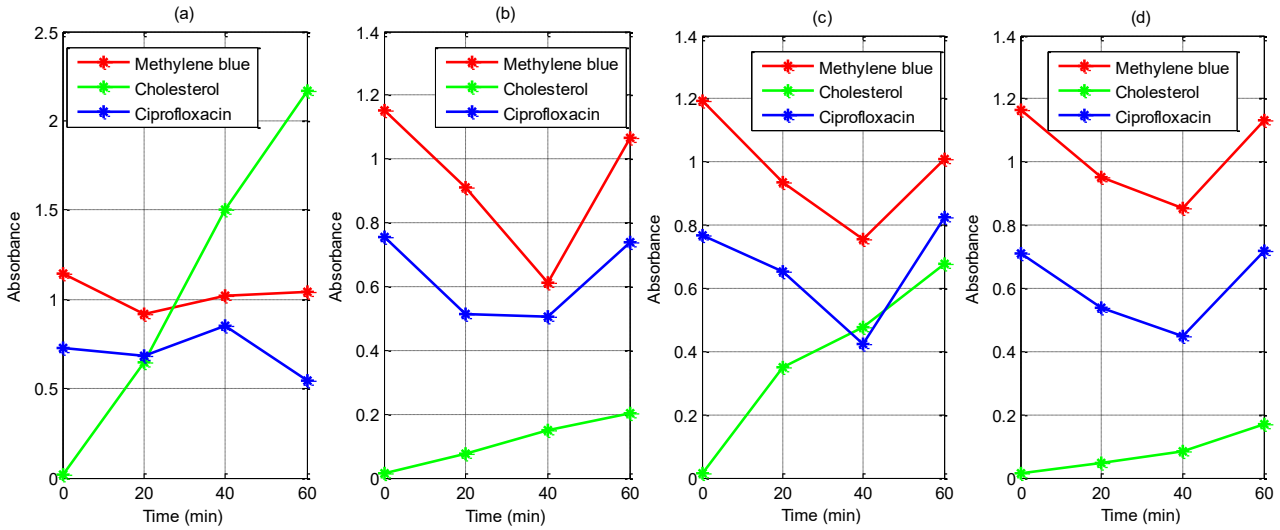

Figure 1: Kinetic curves of pollutant degradation by fungal enzymes (a)FK01, (b)FK02, (c)FK03 and (d)FK04

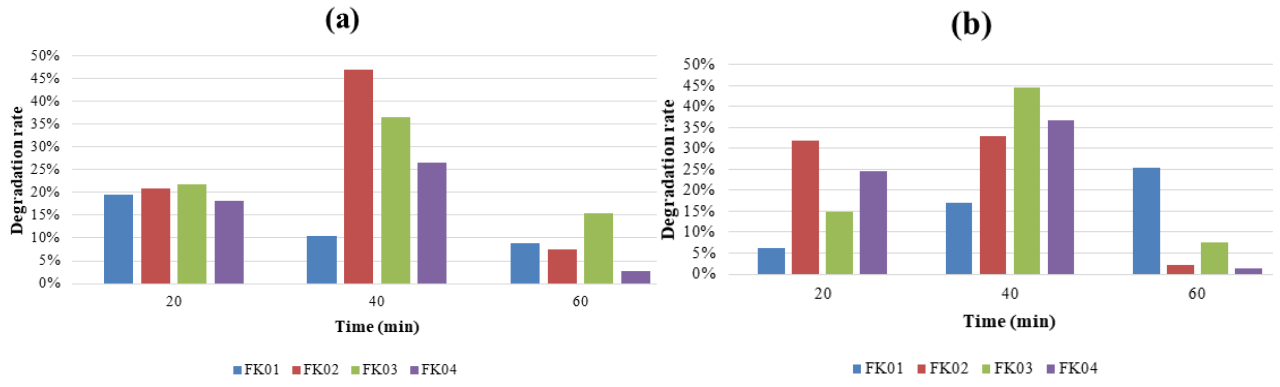

Figure 2: Percentage of degradation by the isolates FK01, FK02, FK03 and FK04 of the different pollutants (a)Methylene blue and (b)Ciprofloxacin

A notable increase in absorbance was observed for Methylene Blue and Ciprofloxacin after 60 minutes of incubation (Figure 2), suggesting the involvement of additional enzymatic processes. Sánchez (2009) described fungal secretomes as complex mixtures of oxidative and reductive enzymes capable of acting simultaneously on substrates. The observed increase may therefore result from reductive enzymes that use cofactors such as NADH or NADPH to convert oxidized intermediates back into their parent or related compounds, thereby influencing spectrophotometric measurements.

Janusz *et al.* further characterized lignin-degrading systems as dynamic redox networks involving both oxidative enzymes (e.g., laccases and peroxidases) and auxiliary reductases (Janusz *et al*., 2017). This interaction creates a metabolic "tug-of-war" within non-purified enzyme systems. Druzhinina *et al*. also emphasized the metabolic versatility of fungal secretomes, which may exhibit synergistic or antagonistic enzyme interactions depending on environmental conditions. Consequently, the observed absorbance fluctuations likely reflect overlapping degradation and re-transformation processes rather than a simple linear pathway.

Cholesterol exhibited a distinct kinetic profile due to its colorimetric assay (Figure 1). Its enzymatic degradation, particularly pronounced in strain FK01, generates hydrogen peroxide (H_2O_2), which oxidizes ABTS into a stable green

radical cation, producing increased absorbance at 500 nm. The high activity observed in FK01 highlights the specialized metabolic capacity of certain basidiomycetes to degrade complex polycyclic molecules, including sterols and lipids.

This capability can serve as a proxy for polycyclic aromatic hydrocarbon (PAH) degradation, as structural similarities enable shared enzymatic pathways. Bilal *et al*. noted that enzymes such as cholesterol oxidases and peroxidases are involved in the breakdown of recalcitrant aromatic pollutants.

Degradation efficiency varied among isolates depending on substrate structure (Figure 2). Methylene Blue was most effectively degraded by FK02 and FK03, while Ciprofloxacin degradation was highest in FK03 and FK04. Cholesterol degradation was most pronounced in FK01, reflecting its metabolic specialization. Overall, these findings demonstrate the metabolic plasticity of the studied fungi and support their potential for mycoremediation of complex contaminated soils.

Preparation and Application of mycoinocula for soil bioremediation

The development of mycoinocula using cereal grains and sawdust as carrier substrates resulted in successful colonization by all fungal isolates after 15 days of incubation at 28°C. Both substrates supported homogeneous mycelial growth; however, cereal grains exhibited faster colonization rates and denser mycelial networks compared to sawdust. This difference is likely due to the higher nutrient availability and readily assimilable carbon sources in grains, which promote rapid fungal proliferation. In contrast, sawdust, being lignocellulosic, supported slower but more structurally stable colonization, consistent with its role as a long-term substrate.

The pre-colonization phase proved essential for establishing a robust inoculum prior to soil application. Fully colonized substrates ensured that fungal biomass was already adapted to solid-state conditions, thereby enhancing survival and competitive ability upon introduction into contaminated soils. This observation is consistent with previous reports indicating that pre-grown fungal inocula improve establishment and degradation efficiency under field conditions (Gupta *et al*., 2017).

Following incorporation into hydrocarbon-contaminated soils, the mycoinocula exhibited distinct biodegradation patterns depending on both fungal strain and carrier substrate. Soils amended with grain-based inocula showed a rapid initial decrease in contaminant levels, likely reflecting the high metabolic activity of actively growing mycelia. In contrast, sawdust-based inocula demonstrated a slower but more sustained degradation trend, suggesting prolonged enzyme release and extended persistence of fungal activity within the soil matrix.

The effectiveness of the bioremediation process highlights the critical role of the carrier substrate as both a physical support and a nutrient reservoir. Beyond facilitating fungal establishment, these substrates may stimulate enzyme production by supplying additional carbon sources, thereby enhancing ligninolytic activity. Furthermore, the incorporation of solid carriers likely

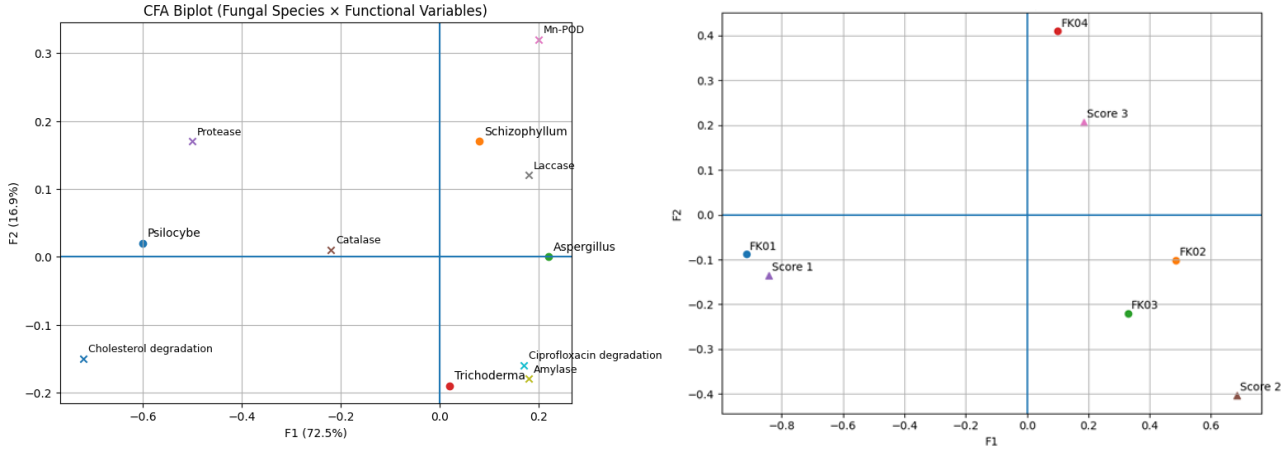

Figure 3: Correspondence Factor Analysis (CFA) of fungal species (a) Functional traits and(b) performance scoring

improved soil structure, aeration, and moisture retention, creating favorable microenvironments for fungal growth and enzymatic degradation.

Periodic monitoring confirmed a progressive reduction of hydrocarbon contaminants alongside the sustained presence of fungal activity in treated soils. This indicates that the introduced strains were capable of surviving, adapting, and maintaining metabolic function under in situ conditions.

These findings demonstrate that the efficiency of mycoremediation is strongly influenced by both the biological characteristics of the fungal isolates and the physicochemical properties of the carrier substrates. The use of pre-colonized grain and sawdust inocula represents a practical and scalable strategy for enhancing the biodegradation of hydrocarbon-contaminated soils.

Statistical analysis

Correspondence analysis (CA) revealed clear functional differentiation among the four fungal strains, accounting for 89.4% of the total inertia (Figure 3). The first axis (F1, 72.5%) represented the principal gradient of variation and separated strain FK01 from the more active strains FK02, FK03, and FK04. FK01 was associated with proteolytic activity, catalase production, and cholesterol degradation, indicating a more specific metabolic profile. In contrast, FK02, FK03, and FK04 were positioned on the positive side of F1, reflecting broader metabolic capabilities.

Among these, FK02 was strongly associated with oxidative enzyme production, particularly laccase and Mn-POD, as well as methylene blue degradation and high growth capacity. FK03 was characterized by hydrolytic activity, notably amylase production, and showed associations with both methylene blue and ciprofloxacin degradation. FK04 occupied a distinct position, combining both oxidative and hydrolytic enzymatic activities with ciprofloxacin degradation and strong growth, indicating a more balanced functional profile.

The second axis (F2, 16.9%) explained additional variability by distinguishing FK02, FK03, and FK04 based on differences in substrate degradation and enzymatic expression. Variable contribution analysis indicated that laccase and Mn-POD were the main factors structuring the distribution of strains, whereas catalase contributed weakly. Cholesterol degradation was primarily linked to FK01.

The ordination pattern highlighted three main groups: FK01, FK02–FK03, and FK04. This grouping reflects differences in enzymatic activity and substrate utilization among the strains. Overall, CA provided a quantitative assessment of the relationships between fungal strains and their functional traits, demonstrating variability in their metabolic profiles.

Conclusion

This study underscores mycoremediation as a promising, economically viable, and low-cost strategy for soil decontamination. By characterizing local fungal strains, we have successfully mapped specific enzymatic profiles to their respective degradation capabilities, offering a roadmap for targeted environmental applications.

Our results demonstrate that the choice of a fungal agent must be tailored to the specific pollutant and environmental context:

- Ligninolytic Specialization (*Schizophyllum*): This genus is the preferred choice for degrading aromatic compounds and dyes (e.g., methylene blue) due to its high production of laccase and Mn-POD.
- Steroid Bioconversion (*Psilocybe*): Despite slower growth and lower overall enzymatic activity, this genus offers unique avenues for the transformation of steroids and complex lipids, specifically regarding cholesterol degradation.
- Versatile Broad-Spectrum Remediation (*Aspergillus* and *Trichoderma*): Characterized by rapid growth and moderate-to-high enzymatic versatility (including amylase and ciprofloxacin degradation), these genera are ideal for complex matrices or general remediation efforts.

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