# Fungi Remediate Soils Contaminated by War-like Activities

Subjects: Environmental Sciences

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Fungi comprise the largest kingdom of higher organisms on the planet: eukaryotes with complex cell structures and abilities to make tissues and organs. Hyphae filaments have a rigid, complex cell wall and moving protoplasm (cytosol) divided into compartments by cross walls termed septa, allowing cellular components to move through these. The plasma membrane comprises a phospholipid bilayer associated with transmembrane proteins and ergosterol and some enzymes such as integral membrane proteins chitin synthase and glucan synthase. The release of enzymes into the extracellular environment, which many fungal species carry out, and the high contact area between filamentous fungi and the soil make these organisms promising for the degradation or immobilization of pollutants (explosives, metals, metalloids, radionuclides, and herbicides) in soil impacted by War-like activities.

Keywords: fungi ; bioremediation ; explosives ; radionuclides ; toxic elements

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## 1. Mycoremediation and Its Techniques

> "*When looking for nature-based solutions to some of our most critical global challenges, fungi could provide many of the answers*." (State of the World's Fungi 2018 by Katherine Willis, Director of Science, Royal Botanic Gardens, Kew)

Bioremediation, by definition, refers to the cost-effective and environmentally friendly method for the efficient conversion of xenobiotics (toxic and recalcitrant pollutants) into environmentally benign products through the action of natural biological treatments of polluted systems such as land and water [1]. Biological agents such as animals, plants, fungi, bacteria, and other organisms, whether naturally occurring, adapted, or modified, have their biochemical capabilities directed toward the removal or transformation/attenuation of environmental pollutants [2][3].

Fungi and their morphologic, physiological, and metabolic characteristics are involved in the conversions of organic and inorganic compounds. Fungal degradative activities (also named mycotransformation or mycodegradation) have been recognized in many circumstances when these microorganisms break down different types of wood, stored paper, textiles, plastics, leather, electro-insulating materials, and various wrapping materials [4]. Therefore, mycoremediation is the bioremediation division that employs fungi to degrade, restore, and heal contaminated ecosystems [1][4].

The substantial contribution of these living organisms to various fields of biotechnology can be tracked through records obtained via bibliographic surveys employing the term "fungal biotechnology", with 13,187 publications distributed across categories in the Web of Science, including *Biotechnology Applied Microbiology*, *Environmental Sciences*, and *Soil Science*. When employing the terms "fungal remediation", "fungal bioremediation", and "mycoremediation", directing attention to fungi in pollutant remediation, it is possible to observe the increasing trend in publications since the 1990s as an eco-friendly and practical approach (**Figure 1**). Thus, the use of the term "mycoremediation" in research on bioremediation is still in its emerging stages. Yet, it mirrors the upward trajectory in utilizing these microorganisms to remediate polluted environments.

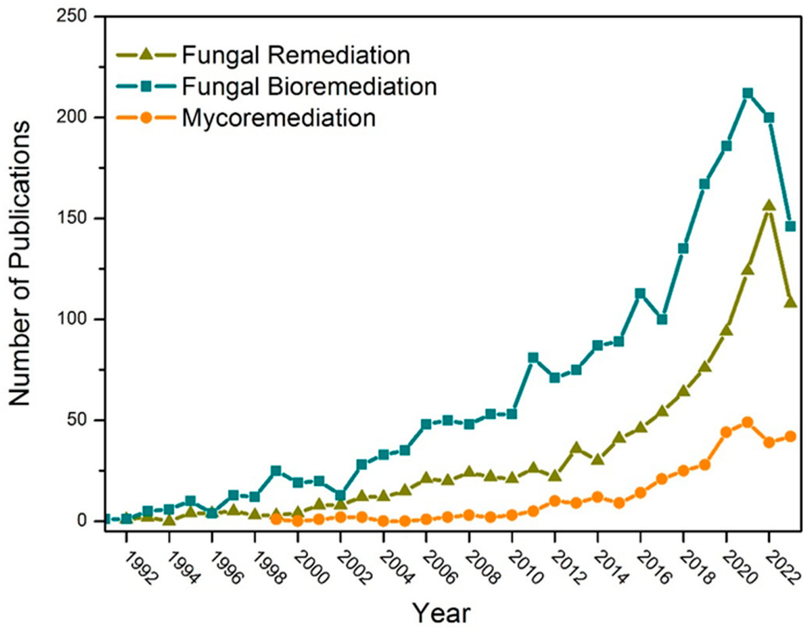


**Figure 1.** Representation of publication trends for "fungal remediation", "fungal bioremediation", and "mycoremediation" between 1990 and 2023 by Web of Science (www.webofscience.com/ (accessed on 30 October 2023).

Despite the magnitude of 120,000 fungi found globally, only a few species have been associated with mycoremediation [4][5]. Principal genera of fungi include *Aspergillus, Cryptococcus, Curvularia, Drechslera, Fusarium, Lasiodiplodia, Mucor, Penicillium, Rhizopus*, and *Trichoderma* [5]. White-rot fungi, including *Phanerochaete chrysosporium*, *Trametes versicolor*, *Bjerkandera adjusta,* and *Pleurotus* sp., are also main agents of the biodegradation of ligninous material and have demonstrated bioremediation potential by different ligninolytic enzyme actions [1][4]. Moreover, due to lifestyle conditions, marine, extremophilic, and symbiotic fungi (mycorrhiza and endophytes) are potential candidates for diverse bioremediation applications [5][6][7].

Fungi can be employed to promote treatment in several matrices polluted with polycyclic aromatic hydrocarbons (PAHs) [8], petroleum hydrocarbons [9][10][11][12][13], biphenyls [14], phthalates [15], polychlorinated herbicides such as polychlorinated dibenzo-p-dioxins–PCDD [16], chlorinated insecticides and pesticides [17], textiles dyes [18], pharmaceutical substances like antibiotics sulfonamides [19], and norfloxacin [20]. Toxic metals, including cadmium, copper, mercury, lead, manganese, nickel, zinc, and iron, and metalloids, with an emphasis on arsenic, are extensively used in different types of industries, being released in high amounts to their effluents, causing direct or indirect environmental contamination where fungi can act to remediate [21][22][23][24][25]. Excellent books and original and review articles describe how these pollutants have been remediated, and some of them are documented in this manuscript as recommended readings, given the specific nature of the treatment for each type of wastewater [1][4][5][6][24][26][27][28][29].

Two primary positive considerations can be observed to support the use of fungi in the remediation of environmental contaminants as opposed to traditional physicochemical processes, namely, they are low-cost and environmentally acceptable, in line with principles of green chemistry [30]. Filamentous fungi can produce a large amount of biomass during their growth and development, which is cheap and readily available as a byproduct of several essential economic fermentation processes [31]. This robust growth of fungus produces a vast hyphal network with a high surface area-to-volume ratio, extracellular ligninolytic enzyme production, resistance to toxic metals, and adaptability to pH and temperature variations, endorsing their use [6].

Several bioremediation technologies are employed to remove or stabilize pollutants from contaminated environments by either applying bioremediation at the point of contamination—named "in situ" bioremediation—or transferring contaminated material (soils, for example) to a remote treatment facility—the "ex situ" methodology [5]. In situ processes include biosparging, biostimulation, bioaugmentation, bioventing, natural attenuation, bioslurping, bioleaching, phytoremediation, and mycoremediation techniques and ex situ methods comprise biopiling, composting, land farming, the use of biofilters, and the use of and bioreactors [5][21]. However, the understanding adopted in this manuscript is that mycoremediation and phytoremediation are used in not just one of the bioremediation techniques but indeed are rather expansive as they play a fundamental role in most of the bioremediation techniques, being applied isolated or in a combination as biological agents of remediation. Thus, fungi are considered as one of the biological agents that perform remediation. **Figure 2** introduces a flowchart of how fungi perform through their mechanisms in these different bioremediation techniques.

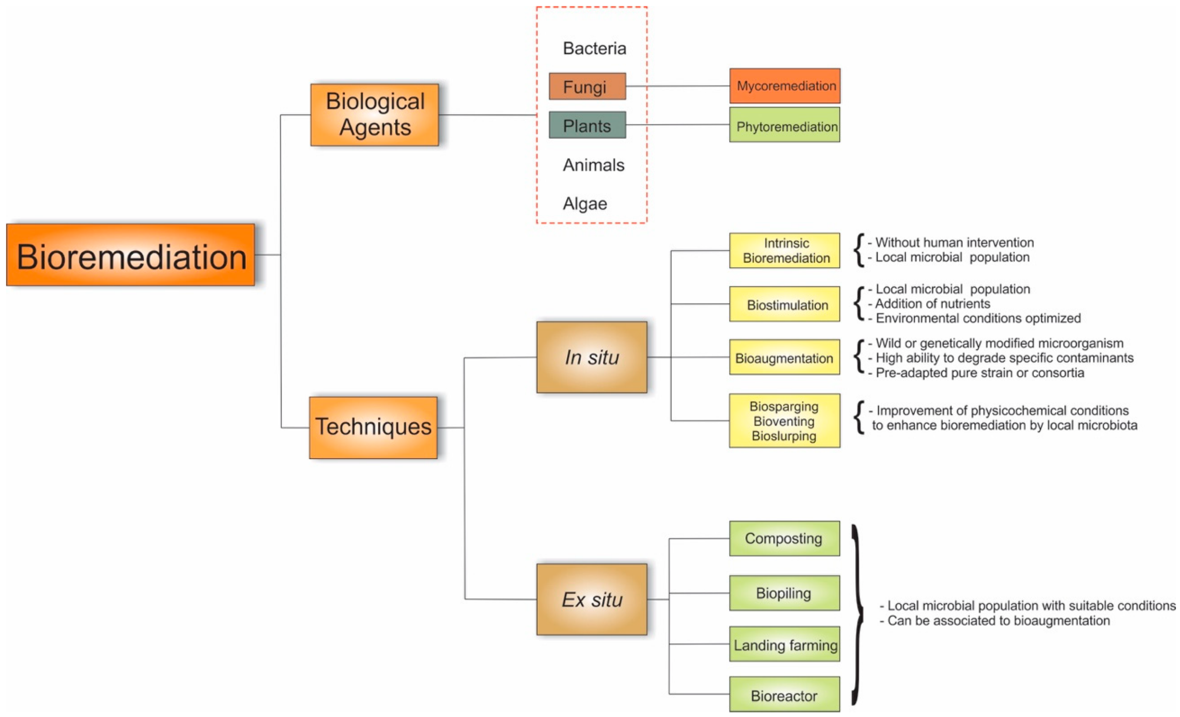


**Figure 2.** Mycoremediation techniques to remove pollutants from contaminated environments.

Intrinsic bioremediation and biostimulation are based on optimizing the local microbiota's metabolism. The former is a natural attenuation process conducted in situ using the inherent propensities of the local microbial population to convert environmental pollutants into nontoxic forms without human intervention [32][33]. Biostimulation exploits the intrinsic ability of local microbiota to succeed in contaminated environments by adding nutrients (carbon, phosphorus, nitrogen) and optimizing environmental conditions (temperature, pH). These conditions improve the growth rate of degrading microorganisms, increasing biomass and expanding their metabolic activity [34][35].

Bioaugmentation is a technique that uses specific microorganisms with high resistance and a good capacity to degrade specific contaminants rapidly. They may be a microorganisms with or without genetic modification, and they are introduced into the treatment area as a pre-adapted pure culture or consortium [32][36].

Biosparging, bioventing, and bioslurping techniques aim to enhance physicochemical conditions to intensify the bioremediation process of the indigenous microbiota. In biosparging, volatile components migrate to the unsaturated zone when air is injected and undergo biodegradation. In bioventing, the stimulation of airflow is regulated. In bioslurping, bioremediation occurs through a combination of bioventing with vacuum-enhanced pumping [5].

On the other hand, ex situ remediation's fundamental goal is to introduce suitable nutrients and humidity and oxygen conditions to native microorganisms using five techniques: composting, biopiling, land farming, using a biofilter, and using a bioreactor. During composting, contaminated soil is placed in treatment containers, mixed with biomass (compost), and aerated during composting by combining the elements for a few weeks [16]. Composting is an age-old strategy for remediating soils contaminated with organic pollutants, such as PAHs, by employing lignin-degrading fungi, including the white-rot fungus *Phanerochaete sordida* [37]. This type of bioremediation can be combined with bioaugmentation to remediate soil and sediment metals. Huang and colleagues [38] investigated the impact of inoculating the fungus *Phanerochaete chrysosporium* on Pb's bioavailability and the bacterial community's diversity in a compost pile of agricultural residues contaminated with this metal.

In the biopiling technique, the soil is arranged in piles to enable the control of leachate from these piles, involving the addition of nutrients or minerals and the injection of air. This method remediates soils contaminated with petroleum hydrocarbons, PAHs, polychlorinated biphenyls (PCBs), and pharmaceutical wastes [32][39]. The basis of landfarming is the controlled application of waste on a soil surface to allow the natural microbiota to biodegrade the contaminants aerobically, such as drilling wastes and refinery waste materials. It involves placing contaminated material, aeration, watering, fertilizing, and removing treated material [32].

The soil slurry reactor process involves mixing contaminated water, soil, and other additives (essential nutrients and oxygen) in a bioreactor to allow the native microorganisms to break down the pollutants [5]. However, this methodology is not famous for contaminated land bioremediation since large amounts of water are required [32].

Therefore, in all bioremediation techniques, one can observe the considerable activity of the native microbiota or the addition of microorganisms capable of rapidly degrading contaminants within their matrices. Fungi are among these microbial agents involved in all bioremediation processes. Mycoremediation mechanisms are complex and offer varied possibilities to reduce the bioavailability of pollutants or degrade them. The following section describes these mechanisms, emphasizing fungi's relevance to decontaminating soils impacted by war-like activities.

## 2. Mycoremediation Mechanisms

Regardless of the bioremediation technique employed to treat pollutants, fungi can partially transform or break down the contaminants into simpler forms and utilize them as substrates for their growth by operating through multiple mechanisms distributed into two categories: biosorption and bioaccumulation.

Biosorption, first introduced in 1951, is defined as the removal/binding of different kinds of organic compounds (such as organic solvents, synthetic dyes, herbicides, insecticides, and pesticides) and inorganic materials (like metals, metalloids, and radionuclides) in their soluble or insoluble forms from an aqueous solution [40][41][42]. The term sorption includes absorption and adsorption: absorption is the inclusion of a substance in one form into another different form, while adsorption is the physical attachment of molecules/ions on a solid material's surface by bonding [43].

In this context, the remediation process for pollutants by fungi can begin through physicochemical interactions between the toxic agents (biosorbate) and the fungal cell wall surface (biosorbent). These interactions depend on the biosorbate's properties, such as solubility, molecular or ionic size, reactivity, and hydrophobicity, and the biosorbent's surface charge and chemical composition [44]. Typically, fungal cell walls are composed of glucans, chitin, mannans, galactomannans, glycoproteins (mannoproteins, galactomannan proteins, and glycosylphosphatidylinositol–GPI anchor), "nonintegral cell wall proteins" (heat-shock, glycolytic enzymes, and hydrophobins), and lipids [30]. These biocompounds comprise numerous functional groups, like the carboxyl (-COOH), carboxylate (-$COO^-$), hydroxyl (-OH), amino (-$NH_2$), thiol (-SH), methoxy (-$OCH_3$), phosphate (-$OPO_3^-$), and sulfate (-$OSO_3^-$) groups, as well as esters (-COOR) and amides (-$CONH_2$). These functional groups play a pivotal role in biosorption through adsorption by engaging in electrostatic interactions and van der Waals forces.

### 2.1. Fungal Biosorption

Although living organisms invariably perform biosorption, the term "biosorption" is primarily employed to denote bioremediation using dead/inactive biomass. It is described as a physicochemical event characterized by a passive and metabolically independent process that occurs rapidly, akin to conventional adsorptive or ion exchange methods [45]. Mycosorption results from the attraction of functional groups on the fungal cell wall, which occurs through a physical or chemical process and depends on the fungal biomass [5]. *Phialomyces macrosporus*, for example, significantly reduced Cd and Pb from synthetic aqueous media by biosorption mechanisms, with a reduction of more than 80% [46].

The biosorption process by inactive biomass can occur through various mechanisms such as physical adsorption, precipitation, ion exchange, and complexation [5][47]:

(a) Physical adsorption: functional groups in the cell wall interact electrostatically and through van der Waals forces with pollutants.

(b) Precipitation: precipitation or solidification is the process of transforming, for example, the toxic metal compounds into their precipitate form, which is less poisonous and almost negligible [48].

(c) Ion exchange: based on the ion exchange mechanism between the sorbent and the studied pollutants through the replacement (exchange) of protons from the exchangeable sites present on the biosorbent surface with contaminants (e.g., metal ions); this mechanism is facilitated by the existence of hydroxyl, carboxyl, and phenols groups [44].

(d) Complexation: functional groups in the cell wall provide the ligand atoms necessary to form complexes with metal ions, which attract and retain metals in the biomass [5]. The formation of surface complexes involves the interaction of pollutants (e.g., metal ions) with oxygen donor atoms from the oxygen-containing functional groups (coordination) [5].

### 2.2. Fungal Bioaccumulation

Bioaccumulation can be understood as a biotransformation process involving an active organism's retention and concentration of a substance or metal [49]. In the case of metals, bioaccumulation occurs as the metal is transported across the membrane into the cell's cytoplasm, where the sequestered metal becomes immobilized within the cell. It is a complex process divided into two stages. As previously mentioned, biosorption occurs in the first stage of rapid chelation of the metal to the cell wall, which is independent of metabolism. The second stage is characterized by the active transportation of metal ions into cells across the cell membrane [49][50].

The mechanism for pollutant removal using live fungal cells, which is metabolism-dependent, can occur extracellularly—on the cell wall surface—or intracellularly. Bioaccumulation mechanisms involve enzymatic and non-enzymatic processes in both extracellular and intracellular modes. The Fungal Biodegradation and Biotransformation section will briefly discuss the enzymatic processes.

The non-enzymatic processes include accumulation inside the cell via active (transport systems) and/or passive (diffusion) uptake mechanisms, exclusion by a permeability barrier, adsorption on extracellular structures (cell wall, capsule, slime), extra- and intracellular precipitation, efflux pumps, and the chelation of metal and metalloids [24]. Fungal biomineralization or bioprecipitation counteract the toxic compounds by oxalate production, a critical metabolite that plays a significant role in many metal and mineral transformations mediated by fungi [51].

In bioaccumulation, metallic species can bind to proteins, leading to precipitation and insertion into specific cellular structures or organelles [52][53][54][55]. Cells form complexes with the undesired metal and sequester it within intracellular organelles for eventual export through efflux systems. Toxic effects can occur, such as the deterioration of biomolecules, leading to changes in cellular properties.

**Fungal Biodegradation and Biotransformation**

Fungi are notably recognized for their decomposer roles with many extracellular and intracellular enzymes, which can be included in biodegradation and biotransformation strategies for remediating polluted areas. Biodegradation happens when filamentous fungi produce extracellular, non-specific, lignin-modifying enzymes, laccases, peroxidases, cellulases, xylanases, amylases, proteases, lipases, and catalases with the capacity to transform pesticides, dyes, and other organic compounds by hydrolyzing polymeric compounds [1][13][51][56]. Inside the cell, intracellular enzymes catalyze diverse reactions, resulting in the biotransformation of toxic compounds, including chemical reactions such as oxidation (cytochrome P 450–CYP 450 monooxygenases, glutathione transferases, oxidative coupling products, hydroxylation), reduction, hydrolysis, alkylation, dehalogenation, transferases (S-transferase, GST) $CO_2$ emission, and others [51][57]. The microorganisms degrade these compounds with the help of endo- and exoenzymes by utilizing them as carbon sources, clearing the toxic compounds from the environment [58].

White-rot fungi are ligninolytic fungi with extensive branching, enabling them to spread in the environment and efficiently access pollutants. They secrete various extracellular ligninolytic enzymes, such as laccase, lignin peroxidase, and manganese peroxidase, that are used not only for the degradation of lignin but also for other pollutants [1][6][51]. For example, a white-rot fungus, *Trametes versicolor*, has been used to treat effluents from the industries of pulp and paper, food, textile, biofuel, cosmetics, and synthetic chemistry [58]. *Phanerochaete chrysosporium*, a white-rot fungus, is acknowledged for remediating organic pollutants [59][60], and *Phanerochaete sordida* was used to treat creosote for contaminated soil [37]. Here, fungi's potential remediation mechanisms to address environmental pollutants are briefly described. These are well substantiated by the various scientific articles supporting this manuscript. **Figure 3** is an overview of the perspective of this manuscript on the various pathways through which fungi play a role in remediation.

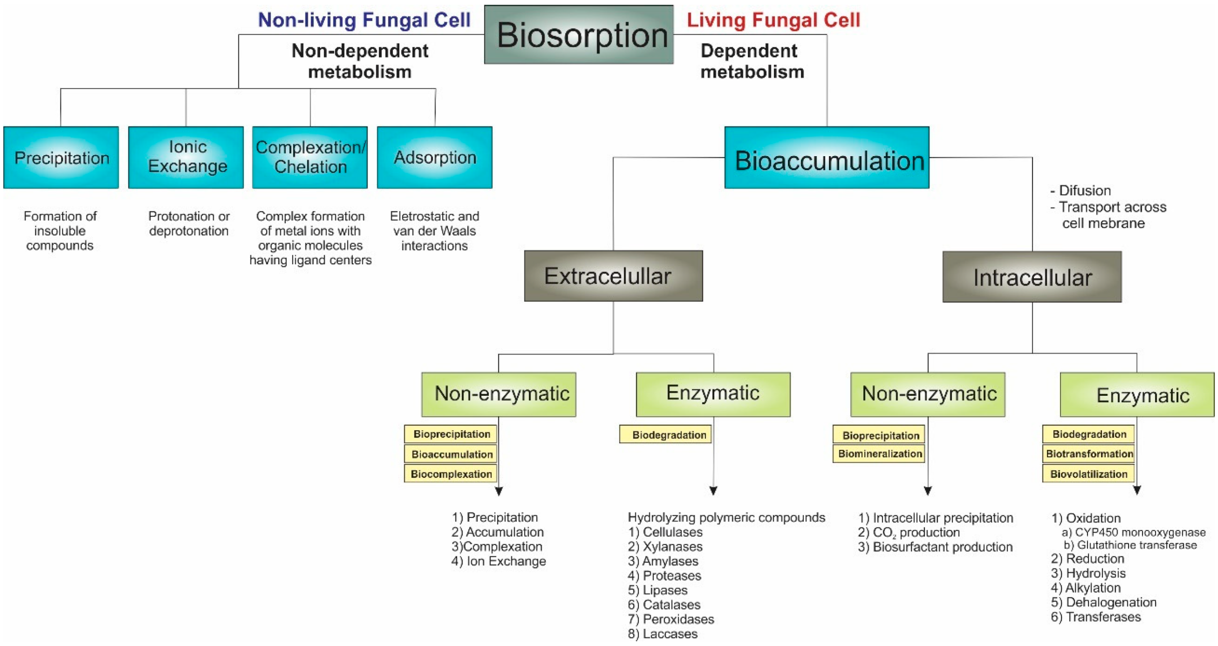


**Figure 3.** Adopted mechanisms of mycoremediation of environmental pollutants (organic compounds, radionuclides, and toxic metals and metalloids).

Living fungal cells have stood out in the mycoremediation of toxic metalloids, radionuclides, herbicides, and explosives. The preference for living fungi highlights the environmental advantages of degrading the structure of organic pollutants (explosives and herbicides) instead of transferring them to another phase, as in purely adsorptive processes. Even concerning metalloids and radionuclides, which cannot be degraded, living fungi can effectively and safely immobilize these pollutants in environments such as soil.

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