Mushrooms as a Biological Tool in Mycoremediation of Polluted Soils

Monika Thakur

Abstract One of the major environmental problems faced by today's world is the contamination of soil, water, and air by toxic chemicals, and the distinct and unique role of microorganisms in the detoxification of polluted soil and environments is well recognized. Fungal mycelia have been primary governors for maintaining ecological equilibrium because they control the flow of nutrients. The strength and health of any ecosystem is a direct measure of its main components—the fungal populations and their interaction with other organisms such as plants, animals, and bacteria. Using fungi as the starter culture species in a mycoremediation project sets the stage for other organisms to participate in the rehabilitation process. The introduction of fungal mycelium into a polluted site triggers a flow of activity and begins to replenish the polluted ecosystem. Mycoremediation is an economically and environmentally sound alternative for bioremediation. It is not widely used at present, but this technology has wider potential than other technologies. Fungi perform a wide variety of functions in ecosystem and potentially have been proven to be clean, simple, and relatively inexpensive for environmental remediation. Examples of fungi used as mycoremediators are *Pleurotus ostreatus; Rhizopus arrhizus; Phanerochaete chrysosporium and P. sordida;* and *Tramates hirsuta and T. versicolor; and Lentinus edodes and L. tigrinus. Thus, this clean technology has greater potential and its untapped potential has to be fully exploited.*

Keyword Bioremediation · Mycoremediation · In situ · Ex situ · Mushroom mycelium · Polluted soil · Underexploited

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M. Thakur (✉)
Amity Institute of Food Technology, Amity University, Noida, Uttar Pradesh, India
e-mail: mthakur1@amity.edu

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1 Introduction

One of the major environmental problems faced by today's world is the contamination of soil, water, and air by various toxic chemicals as a result of industrialization and extensive use of chemicals in agriculture. There are various "**clean technologies**" which emphasize on reduced waste generation, treatment and conversion of waste into some useful form. These clean technologies emphasize on the use of various biological methods for the remediation of waste. The soil is getting more and more polluted day by day. Remediation of the polluted soils is a challenging job. **Bioremediation** is a treatment process that uses naturally occurring microorganisms to break down, or degrade, hazardous substances into less toxic or nontoxic substances. Bioremediation is an attractive technology that utilizes the metabolic potential of microorganisms in order to clean up the environmental pollutants to the less hazardous forms with less input of chemicals, energy, and time (Asgher et al. 2008; Haritash and Kaushik 2009).

The microbes used to perform the function of bioremediation are known as **bioremediators**. "To bioremediate" means to use living things to reclaim contaminated environments. The introduction of exogenous microorganisms into environments—**bioaugmentation**, has been used as an attempt to accelerate bioremediation (Watanabe 2001). Some microorganisms that live in soil and groundwater naturally eat certain toxic chemicals that are harmful to the environment. Watanabe (2001) reported that many naturally occurring microbes have been utilized in a variety of bioremediation processes.

One such biological clean technology is "mycoremediation" which is based on the use of fungi for the removal of waste and toxins from the environment. **Mycoremediation** is one of the most successful and technical areas of bioremediation, which refers specifically to the use of fungal mycelium (Singh 2006). It is a process of using fungi to degrade the contaminants in the polluted environment. Jagtap et al. (2003) discussed mycoremediation as a bioremediation process of using fungi (saprophytic, parasitic, and mycorrhizal) to remove pollutants from the environment. Fungal mycelium stimulates microbial and enzyme activity, and thus reduces in situ production of toxins. The potential applications for mycoremediation technologies have been reported from time to time. Fungal species have been shown to accumulate toxic metals, and even rare earth elements. Fungi are great biodegrades and the resultant compost has been used to enhance the growth of plants as well as bioremediation activity in the environment (Jagtap et al. 2003). Mycelia of fungi are unique among microorganism having the ability to enhance plant growth. They secrete variety of extracellular enzymes involved in pollutants degradation. Some fungi are hyperaccumulators, and are capable of absorbing and concentrating heavy metals in the fruiting bodies of mushrooms (Jagtap et al. 2003). Mycoremediation process involve mixing of mycelium into contaminated soil, placing mycelial mats over toxic sites, or and even the combination of these two techniques. Mycoremediation has been applied to oil spills, contaminated and polluted soil, industrial chemicals, contaminated water, and even farm wastes (Bennet et al.

2001). Bioremediation technology leads to degradation of pollutants and may be a lucrative and environmentally beneficial alternative (Thakur 2015).

Macrofungi (mushrooms) and other microfungi possess enzymes for the degradation of variety of pollutants (Purnomo et al. 2013; Kulshreshtha et al. 2013). However, mushrooms are becoming more popular nowadays for bioremediation process because they are not only a good bioremediation tool but also provide nutritional and health benefits (Kuforiji and Fasidi 2008; Zhu et al. 2013; Thakur 2014; Thakur 2015). Their multidirectional role has now attracted researchers to work in the field of mushroom cultivation and mycoremediation.

In the present scenario, the pollution is increasing at a faster pace. The content of toxic and heavy metals in the environment is increasing day by day and is emerging as a serious problem. In the past, various other treatment methods like thermal, chemical, and physical have failed to reduce or eliminate the pollution problem because those methods can only shift the pollution to a new phase, i.e., air pollution and make air more polluted (Williams et al. 1992). The simultaneous cleanup of all these contaminants by thermal/chemical/conventional method is technically difficult and expensive, and these very methods can also destroy other soil biotic components (Dua et al. 2006). Therefore, mycoremediation is an emerging cleanup technology for polluted sites. Keeping this in mind, the chapter discuss the use of mushroom species as a biological tool for cleanup and degradation of waste and pollutants present in the environment. This chapter aims to study the role of macrofungi and exploit their potential to remediate the polluted soil.

2 Fungi Role in Mycoremediation

Fungi have been used in many diverse applications since ancient times. They have been the major decomposers of various complex polymers as cellulose, hemicellulose, and lignin in the ecosystem (D'Annibale et al. 2006). Macrofungi also plays an important role as natural environment remediator (Pletsch et al. 1999; Matsubara et al. 2004; Thakur 2015). Mycoremediation is an innovative biotechnological application that uses fungus for in situ and ex situ cleanup and management of contaminated sites (Thomas et al. 2009). Mushroom has been used for nutritional and medicinal purposes since times immemorial. Fungi have been nature's most powerful decomposers, secreting strong enzymes. They have also known as mycoremediation tool because of their use in bioremediation of different types of pollutants. They have fast mycelial growth, great biomass production and extensive hyphae which will grow and reach deeper components of environment. This clean technology is based on efficient enzymes (cellulases, hemicellulases, lignin-degrading enzymes, etc.) produced by various mushroom species. These fungi have also been known to transform a wide variety of hazardous pollutants by the process of bioremediation (Alexander 1994; Ashoka et al. 2002).

Fungal mycelia have been primary governors for maintaining ecological equilibrium because they control the flow of nutrients. The strength and health of any ecosystem is dependent upon basic thing—the fungal populations and their interaction with living organisms (producers, consumers, and decomposers). The introduction of fungal mycelium as "starter culture" into a polluted site triggers a flow of enzymes and begins to replenish the polluted ecosystem.

Mycoremediation is an economically and environmentally sound alternative for bioremediation. It restores depleted and polluted soils. Currently, burning, hauling, and constructing new buildings have been the common practices to remove or clean up toxic wastes. But because of these processes, environments do not get rid of all the waste materials and slowly but steadily the pollutants have been added to back to the soil only. This leaves the soil lifeless and contaminated. Various toxins (including mercury, PCBs, and dioxins) are added to our food chain, and become more concentrated at each and every step. Fungal mycelia can destroy these toxins in the soil before they enter our food supply chain. Mycoremediation is thus a biological mechanism to destroy, transform, or immobilize environmental contaminants (Adenipekun and Lawal 2012).

Mycoremediation is not widely used at present, but this technology has wider potential. Fungi perform a wide variety of functions in ecosystem and potentially prove to be clean, simple, and relatively inexpensive for environmental remediation (Kulshrestha et al. 2014). Loske et al. (1990) reported the main contaminants of polluted soils mainly polycyclic aromatic hydrocarbons (PAH's); polychlorinated biphenyls (PCB's), and dioxins.

3 Process of Mycoremediation

Mushrooms use different methods to decontaminate polluted sites and stimulate the environment, such as biodegradation, biosorption, and bioconversion.

3.1 Biodegradation

The biodegradation mechanism is very complex process. In this process, there is degradation and recycling of complex molecules to its mineral constituents. The term "*Biodegradation*" is used to describe the ultimate degradation and recycling of complex molecule to its simpler mineral constituents. It is the process which leads to complete mineralization of the complex compound to simpler ones like CO_2, H_2O, NO_3, and other inorganic compounds by living organisms. Table 1 enlists the enzymes secreted and degradation abilities of various mushroom species. Mushrooms produce various extracellular enzymes such as peroxidases, ligninase (lignin peroxidase, manganese-dependent peroxidase, and laccase), cellulases, pectinases, xylanases, and oxidases. These enzymes are induced by their substrates and are capable of degrading nonpolymeric, recalcitrant pollutants such as

Table 1 Role of mushroom species in biodegradation of pollutants

Sr. No.	Mushroom Species	Role as a mycoremediator	References
1	*Pleurotus ostreatus*	Oxo-biodegradable plastic: mushroom species growing on plastic degraded the plastic.	da Luz et al. (2013)
2	*Lentinula edodes*	Mushroom species degraded 2,4-dichlorophenol (DCP) by using vanillin as an activator.	Tsujiyama et al. (2013)
3	*Pleurotus pulmonarius*	Radioactive cellulosic-based waste: Waste-containing mushroom mycellium was solidified with cement and then the solidified waste acted as the first barrier against the release of radio-contaminants from the site.	Eskander et al. (2012)
4	*Auricularia sp.*, *Schizophyllum commune,* and *Polyporus* sp.	Malachite green dye was degraded by the mushroom species in 10 days.	Rajput et al. (2011)
5	*Pleurotus pulmonarius*	This mushroom species helps in the degradation of crude oil.	Olusola and Anslem (2010)
6	*Coriolus versicolor*	This mushroom species possesses the ability to degrade PAH with the help of lignin-modifying enzymes laccase, manganese-dependent peroxidase (MnP), and lignin peroxidase (LiP).	Jang et al. (2009)

nitrotoluenes, PAHs, organic and synthetic dyes, pentachlorophenol (VanAcken et al. 1999; Hammel et al. 1991; Johannes et al. 1996; Ollikka et al. 1993; Heinfling et al. 1998; Lin et al. 1990; Haritash and Kaushik 2009) under in vitro conditions. Recently, it has also been reported that various mushroom species are also able to degrade polymers such as plastics (da Luz et al. 2013).

3.2 Biosorption

Biosorption is the process for the removal for the removal of pollutants from the environment with the help of mushroom. It has been considered as an alternative to the remediation of industrial effluents as well as the recovery of metals present in effluent. Biosorption is a process based on the sorption of metallic ions/pollutants/xenobiotics from effluents by live or dried biomass which often exhibits a marked tolerance towards metals and other adverse conditions.

Several chemical processes may be involved in biosorption like adsorption, ion exchange processes, and covalent binding. The polar groups of proteins, amino acids, lipids, and structural polysaccharides (chitin, chitosan, glucans) may be involved in the process of biosorption. Table 2 enlists the biosorptive capacity of biomass of mushroom species. They also reported that the biosorption capacity of dead biomass is greater/similar to/less than that of living cells.

Table 2 Removal of pollutants by biomass of mushroom using biosorption process

S. no	Mushroom species	Pollutants and role of mushroom species	References
1	*Agaricus bisporus*, *Lactarius piperatus*	Cadmium (II) ions*: mushroom species* showed higher removal efficiency on Cd(II) ions	Nagy et al. (2013)
2	*Fomes fasciatus*	Copper (II): Mushroom is efficient in biosorption of Cu (II) ions	Sutherland and Venkobachar (2013)
3	*Pleurotus platypus, Agaricus bisporus, Calocybe indica*	Copper, zinc, iron, cadmium, lead, nickel: Mushroom species are efficient biosorbents for the removal these ions from aqueous wastes.	Lamrood and Ralegankar (2013)
4	*Flammulina velutipes*	Copper: Mushroom fruiting body used as biosorbents for removing copper ions from aqueous wastes.	Luo et al. (2013)
5	*Pleurotus tuber-regium*	Heavy metals: *Mushroom species* bioabsorbed pollutants (heavy metals) from soils artificially contaminated with some heavy metals.	Oyetayo et al. (2012)
6	*Pleurotus ostreatus*	Cadmium: Mushroom species bioabsorbed cadmium ions from the substrate.	Tay et al. (2011)
7	*Pleurotus sajor-caju*	Mushrooms bioabsorbed heavy metals.	Jibran and MilseeMol (2011)

3.3 Bioconversion

In this process there has been conversion of industrial waste into some mushroom species. The lignocellulosic waste, generated by industries, can be used for cultivation of mushroom which can be further used as a product. Mushroom species cultivated on industrial and agroindustrial wastes are given in Table 3 (Kulshreshtha et al. 2010; Kulshreshtha et al. 2013).

4 Potential of Mushrooms in Mycoremediation

Although bioremediation by bacterial agents has received attention of many researchers, the role of fungi has been still inadequately explored. The ability of fungi to transform a wide variety of hazardous chemicals has aroused interest in using them for bioremediation. Mushroom forming fungi are amongst nature's most powerful decomposers, secreting strong extra cellular enzymes due to their mycelial growth and biomass production (Elekes and Busuioc 2010). These enzymes include lignin peroxidases (LiP), manganese peroxidase (MnP), and laccase. Thus, carbon sources such as sawdust, straw, and corncob can be used to enhance degradation rates by these organisms at various polluted sites (Adenipekun and Lawal 2012). *Phanerochaete chrysosporium*, *Agaricus bisporus*, *Trametes versicolor*, *Pleurotus*

Table 3 Bioconversion of waste by mushroom species

S. No.	Waste material	Mushroom species	Mushroom cultivation	References
1	Handmade paper, cardboard, and industrial waste	*Pleurotus citrinopileatus*	Successfully cultivated. Basidiocarps possessed good nutrient content and no genotoxicity	Kulshreshtha et al. (2013)
2	Sawdust of different woods	*Pleurotus ostreatus*	Biomass of mushroom has been produced in submerged liquid culture were analyzed	Akinyele et al. (2012)
3	Agroindustrial residues such as cassava, sugar beet pulp, wheat bran, and apple and pear pomace	*Volvariella volvacea*	Enzyme activities were measured during the fermentation of substrates	Akinyele et al. (2011)
4	Handmade paper, cardboard and industrial waste	*Pleurotus Florida*	Successfully cultivated. Basidiocarps possessed normal morphology and no genotoxicity	Kulshreshtha et al. (2010)
5	Cotton waste, rice straw, cocoyam peels and sawdusts of *Mansonia altissima, Boscia angustifolia*, and *Khaya ivorensis*	*Pleurotus*	Successfully cultivated with good crude protein, fat, and carbohydrate contents in fruiting bodies.	Kuforiji and Fasidi (2009)
6	Paddy straw, sorghum stalk, and banana pseudostem	*Pleurotus eous* and *Lentinus connatus*	Waste successfully bio-converted by mushroom with good biological efficiency	Rani et al. (2008)
7	*Terminalia superba, Mansonia altissima, Holoptelea grandis,* and *Miliciaex excelsa*	*Pleurotus tuber-regium*	Mushroom species grown on trees	Jonathan et al. (2008)
8	Cotton waste, sawdust of *Khaya ivorensis* and rice straw	*Pleurotus tuber-regium*	Sclerotia propagated on groundnut shells and cocoyam peels with lipase and phenoloxidase; cellulase, carboxy-methylcellulase enzymatic activities	Kuforiji and Fasidi (2008)
9	Eucalyptus waste	*Lentinula edodes*	Successfully converted this waste and qualitative and quantitative changes were also measured	Brienzo et al. (2007)
10	Mushroom fruiting bodies were grown on vineyard prunings, barley straw and wheat straw	*Lentinula edodes*	Bioconversion of waste having highest biological efficiency, yield, and shortest production cycle	Gaitán-Hernández et al. (2006)

(continued)

Table 3 (continued)

S. No.	Waste material	Mushroom species	Mushroom cultivation	References
11	Wheat straw	*Lentinula tigrinus*	Characterized the production of lingocellulosic enzymes and bioconverted the wheat straw into fruiting bodies	Lechner and Papinutti (2006)
12	Banana leaves	*V. volvacea*	Efficient bioconversion with good yield of fruiting bodies	Belewu and Belewu (2005)

ostreatus, and other many mushroom species have been reported for decontamination of polluted sites (Adenipekun and Lawal 2012; Thakur 2015). Mushrooms have long been known for their nutritive and medicinal benefits. Sesli and Tuzen (1999) reported that mushrooms can be used to evaluate the level of environmental pollution and to remediate metal-polluted soils. Also, many studies have been carried out to evaluate the possible threats to human health from the ingestion of mushrooms containing heavy metals (Tismal et al. 2010; Ouzouni et al. 2009; Sesli and Tuzen 1999).

Based on literature and research, white rot fungus accounts for almost 30% of the total research on fungi used in bioremediation process (Adenipekun and Lawal 2012). White rot fungi have been used for bioremediation of pesticides, degradation of petroleum hydrocarbons and lignocellulolytic wastes in the pulp and paper industry. White rot fungi are excellent mycoremediators of toxins held together by hydrogen–carbon bonds. Enzymes secreted by white rotters include lignin peroxidases, manganese peroxidases, and laccases.

Some specific examples of macrofungi mycelium especially white rot fungus used for mycoremediation are (Fig. 1):

Sr. No.	Macrofungi as mycoremediator	References
1	*Phanerochaete chrysosporium*	Leonardi et al. (2007), Adenipekun and Lawal (2012), Sasek and Cajthaml (2005), Nigam et al. (1995), Aitken and Irvine (1989), Bumpus et al. (1985), Barr and Aust (1994)
2	*Lentinus edodes*	Adenipekun and Lawal (2012)
3	*Lentinus tigrinus*	Stella et al. (2012)
4	*Lentinus squarrosulus* (Mont.) Singer	Adenipekun and Fasidi (2005) and Adenipekun and Isikhuemhen (2008)
5	*Pleurotus ostreatus* (Jacq. Fr.) P. Kumm	Sack and Gunther (1993), Bojan et al. (1999), Sykes (2002), Okparanma et al. (2011), Baldrian et al. (2000), Eggen and Majcherczyk (1998), Eggen and Sveum (1999), Bhattacharya et al. (2012)
6	*Pleurotus tuber-regium* (Fries) Singer	Isikhuemhen et al. (2003), Adenipekun et al. (2011a)
7	*Pleurotus pulmonarius*	Adenipekun et al. (2011b)
8	*Trametes versicolor*	Stamets (2010), Tanaka et al. (1999), Novotny et al. (2004), Morgan et al. (1991), Gadd (2001)
9	*Bjerkandera adusta*	Adenipekun and Lawal (2012), Pozdnyakova (2012)
10	*Irpex lacteus*	Bhatt et al. (2002), Adenipekun and Lawal (2012)

Fig.1 (**a**) *Phanerochaete chrysosporium,* (**b**) *Lentinus edodes,* (**c**) *Lentinus tigrinus,* (**d**) *Lentinus squarrosulus* (Mont.) Singer, (**e**) *Pleurotus ostreatus* (Jacq. Fr.) P. Kumm; (**f**) *Pleurotus tuber-regium* (Fries) Singer (**g**) *Pleurotus pulmonarius;* (**h**) *Trametes versicolor;* (**i**) *Bjerkandera adusta;*(**j**) *Irpex lacteus*

5 Advantages of Mycoremediation

Mycoremediation technologies help in fungal species growth and increase its population by creating optimum environmental conditions for them to detoxify the maximum amount of contaminants. A fungus produces various nonspecific enzymes which can act on wide variety of environment pollutants. Hyphae allow fungi to expand their surface area, make them easier to contact the pollutant. There have been numerous advantages of using mycoremediation over commercialized technologies, including the following:

- Public acceptance.
- Natural and environment friendly.
- Safety.
- Simple and quiet.
- Low maintenance.
- Reusable end products.
- Low cost.
- Flexibility.
- Fast.

6 Constraints for Mycoremediation

The use of macrofungi like mushrooms for remediation of polluted soils has been known in the recent years. This mycoremediation is not only a clean technology but also generate fruiting bodies. Thus the mushroom production can generate not only a healthy food but also the food for livelihood. Research has shown that mushroom species like *P. ostreatus* and *P. chrysosoporium* have emerged as model systems for studying bioremediation. But, a great deal still remains to be learned about the basic knowledge of how this white-rot fungus removes pollutants. The majority of mycoremediation work has been done on *Phanerochaete chrysosporium*. The fungus has the potential of mycoremediation because of its lignin-degrading enzyme system. A similar degrading ability has been described by other species of white rot fungus but not with the degree of success reported for *Phanerochaete chrysosporium*. Sasek (2003) reported that the performance of white rot fungus in soil bioremediation depends upon its survival in the soil environment, colonization, relationship and interaction with other soil microflora. Still, research trials on other fungi are unexplored and underexploited.

Mycoremediation is a very important process but still there are various problems that are hindering its widespread use. Boopathy (2000)) discussed some of the factors limiting bioremediation technologies. Various challenges faced are:

- Contamination by other fungi (*Penicillium* spp., *Aspergillus* spp.) while interaction with them.

- Fungal species are unable to compete with other native microbes in soils. Some bacteria could either inhibit the growth of fungi, or in combination with fungi enhance degradation of pollutants.
- Nutrient cycle should be completely understood.
- Starter cultures: The problem to be borne in mind is that in bioremediation projects mushroom mycelium should not be used as a starter material.
- Legal issues: There are also legal issues in this process. There are several patents specifically granted for matching fungus against a toxin. This is a major hindrance in preventing wide-scale fungal cleanup of toxins from polluted sites.
- Mushroom cultivation process: The lack of experienced mushroom cultivators in outdoor trials is a problem in mycoremediation. This lacking has affected the success of several trials.

Therefore, though there are many constraints in this effective technology, it has proven to be a boon to the soil. More research is still required for further exploration.

7 Future Prospects

In recent advancements the addition of required fungal strains to the soil, the enhancement of the indigenous microbial population and its ability to break down various exposures contaminants have proven successful. Whether the fungal mycelia are native or newly introduced to the site, the process of destroying contaminants is important and critical for understanding mycoremediation. There is no definite time frame for complete mycoremediation as the time taken by various contaminants and types of applications will vary. That is why the research in this area is still in the experimental phase and unexplored. Further, the application of this technology in large scale projects will demand much more work to streamline the methodologies.

Once the research and technology is complete, it will have a wider application. With appropriate funding by the government/semi-government and other organizations, the technology could be developed and made available for commercialization. However, current funding has been limited. But extensive research needs to be pursued as the technology has proven successful in micro-sites. This cleaner technology has been expected to be faster and more cost-effective than other remediation technologies once it is commercialized. The use of fungi for remediation would allow commercial concern to offer inexpensive, safe products to their customers. If the underexploited potential of fungus mycelium is further exploited, it will go a long way in eradicating pollution from soils. Thus the mission of the pollution-free environment can be achieved and our future generations will have a better environment to live.

8 Conclusion

Fungi can be used an effective tool to reduce waste materials in contaminated soils via nonspecific enzymes activities. Evidences have shown that mushrooms have the potential to clean up soils contaminated with various toxic elements. Mycoremediation is not a panacea, but an effective and powerful tool to remediate soil pollution. Mushrooms have tremendous potential to be used in bioremediation process. The cultivation of edible mushroom species on agricultural and industrial wastes may thus be a value-added process capable of converting these wastes into foods (mushrooms). Besides producing nutritious mushrooms, it reduces genotoxicity and toxicity of contaminated sites. Mycoremediation through mushroom cultivation will alleviate two of the world's major problems, i.e., waste accumulation and production of proteinaceous food, simultaneously. Thus, there is a need for further research towards the exploitation of potential of the mushroom species as a bioremediation tool and its safety aspects for consumption as a food product.

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