

# Chapter 4

## Mycoremediation of Agricultural Soil: Bioprospection for Sustainable Development



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### Abbreviations

2,4-D	2,4-Dichlorophenoxyacetic acid
AmDNT	Amino-dinitrotoluene
CBA	Chlorobenzoic acids
DDT	Dichlorodiphenyltrichloroethane
LiP	Lignin peroxidase
MnP	Manganese peroxidase
PAH	Polycyclic aromatic hydrocarbons
PCBs	Polychlorobenzoic acids
SVOCs	Semi-volatile organic compounds
TNT	Trinitrotoluene
VOCs	Volatile organic compounds

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## 4.1 Introduction

Soil is a complex interface of biotic and abiotic components comprising of minerals, water, gasses, organisms, etc. Healthy soil nourishes plants, animals, and microbes as well as humans for growth and survival for many centuries till today. However, in the past few decades, widespread exploitation is turning this soil gradually unhealthy and unfit for proper plant nourishment. Intensive use of fertilizers and agrochemicals along with industrialization adds excessive heavy metals and toxic pollutants that become the main obstacle for upbringing and sustaining the life-supporting system in soil. The presence of heavy metals, pesticides, and chemical pollutants in soil and water is the major concern, as they are not degraded easily into nontoxic forms and have long-lasting effects on the environment, which ultimately enter into the agri-food chain. Cancer, kidney failure, mental disorder, and paralysis, along with physical weakness, headaches, diarrhea, and anemia, are the unpleasant consequences of the chronic exposure to these toxic and persistent chemicals. In concern to this alarming situation, many attempts have been made since the mid-1980s to find out the ways to prevent the continuous degradation of soil health due to industrial pollutants and to enhance soil health to a state of nontoxicity.

Although several techniques (viz., chemical precipitation, oxidation-reduction, filtration, ion exchange, dialysis, and electrochemical treatments) are available to remove these heavy metals and pollutants from soil, but none of them is fully effective. Moreover, higher cost, restricted application with limited prospects, and inability to improve inherent soil health make them nearly abandoned. In replacement, bioremediation process emerged as the potential alternative with eco-friendly and cost-effective remediation strategy. Bioremediation is basically a process of reducing contaminant levels to undetectable, nontoxic, or at least acceptable levels (i.e., within limits set by regulatory agencies) by exploiting available natural bioresources, which mostly include plants, microbes, organic amendments, etc. that alter these contaminants.

The term “mycoremediation” was coined by Paul Stamets; and it refers to detoxification of contaminated site by using one or more species of fungi. It is a process of sequestering toxic contaminants from soil and water by using fungi. Biotechnological exploitation of such fungi for in situ and ex situ cleanup and management of contaminated soil and water is considered as mycoremediation (Strong and Burgess 2008). It involves enhancement of soil health using native and/or alien microflora. This type of bioremediation involves specific augmentation of fungal cultures that helps in rapid decomposition. Saprophytic fungi play an important role in the decomposition of organic molecules. These fungi secrete diverse extracellular enzymes and acids that break down natural polymers like keratin, chitin, lignin, pectin, cellulose, and hemicellulose (King et al. 1997; Lamar and White 2001). Besides decomposition, some fungi also produce small amount of biostatic or bio-cidal compounds that are involved in the bioremediation of complex organic and

inorganic molecules like hydrocarbons and heavy metals. Some approaches involve enriching contaminated soils with manure and compost, while others have focused on the mass application of single organism including flora or fauna on or into the soil. Meanwhile others adopted holistic approach to use consortia of plants, fungi, and bacteria. Many of these applications have shown wonderful results in degrading several toxins, pollutants, and hazardous chemicals (Singh 2006). Overall, inclusion of these approaches in combination may be complementary and may maximize the prospect and effectiveness of bioremediation. Partnering fungi for bioremediation open up the door to accelerate soil healing process. They have great potential in mycoremediation by playing early events of degradation and also by supporting other organisms in the remediation activity. Many fungi through the enzymatic degradation of contaminants supply energy to stimulate aggressive toxin degradation by other microbes present in the soil. Moreover, fungi can also remove heavy metals by channeling them into their fruiting bodies; thereby, they perform excellent job in revitalizing rhizospheric ecosystem (Prasad 2017).

Fungi, the eukaryotic microorganisms, are ubiquitous and represent diverse groups from various environments (Deacon 2006). They are well adapted to almost every habitat, from agricultural land to forest ecosystem and from marine to arctic environment. The natural behavior of fungi is to work within community and support a large number of organisms in ecosystem. Fungal community is not disassembled; it always prefers living in association with thousands of other living beings and plays vital role in most of the ecosystems and is proficient in regulating the flow of nutrients and energy. Fungi are widely known for their skill in breaking down advanced plant cell structures and complex cellular molecules like cellulose, hemicellulose, lignin, etc. They are well equipped with highly advanced mechanisms and digestive enzymes for pulling apart and degrading very complex carbon-based compounds into simpler form (Deacon 2006), and thus, they regulate C:N ratio and participate in nutrient recycling in soil. Besides, some species are blessed with mechanisms for degrading toxins, pollutants, and heavy metals (Pointing 2001) and, hence, can be an essential component of remediation process. The most important bioremediation process of soils involves microbial metabolism (Kearney and Kellog 1985). Probably degrading microorganisms obtain carbon, nitrogen, or energy from the pesticide molecules and other toxicants. Similar to other microbes, fungi are also known to degrade or to deteriorate a wide variety of pollutants and toxicants. Through mycelial networks, they may cover several hectares of land and speed up the process of soil reclamation. They have the inherent capacity to break down molecules, disassembling long-chained toxins into simpler and less toxic chemicals, and, hence, are considered natural ecosystem engineer (Lawton and Jones 1995) and can be used to create pollutants-free soil environment through the process of mycoremediation (Prasad 2017). Although many reviews exist on mycoremediation, but almost all of them focus on reclamation of soil from hazardous pesticides and herbicides. However, in this chapter, our main emphasis is on the various aspects of mycoremediation, specifically bioprospection of fungi and their role in maintenance/improvement of soil health.

## 4.2 Concepts of Bioremediation

Bioremediation is a natural process of decontaminating soil and groundwater from organic pollutants using living organisms (Rhodes 2014). It involves the techniques of waste management for eliminating pollutants from the environment in effective and eco-friendly way (Kumar et al. 2011). These hazardous wastes can be removed or detoxified by various living organisms mostly, plants, fungi, and bacteria which are also known as bioremediators. The main purpose of bioremediation is to abate or clean up soil environment from contamination and to improve soil health through eco-friendly approaches. The various approaches of bioremediation can be categorized in two types, in situ and ex situ (Azubuike et al. 2016). In situ bioremediation approach involves treatment of intoxicated soils right at the point of contaminated sites itself and provides facility for avoiding excavation and transportation of contaminants; hence, there is no chance of spread of toxicants during excavation (Talley 2005). However, ex situ bioremediation approach involves excavation of contaminated soils and water from sites and subsequent transportation to another site for treatment and makes them free of toxicants and pollutants. Both the approaches include various processes that are briefly discussed below.

### 4.2.1 *In Situ Bioremediation Approaches*

It is a purely biological transformation process, in which fungal enzymes act as catalyst for breakdown and cleaning of the hazardous substances present on the subsurface. During the process of in situ bioremediation, organic contaminants are degraded and used by certain microbes for their growth, providing the supply of macro-(nitrogen, phosphorus) and micro-nutrients (sulfur and trace elements), and energy. In most cases, organic compound containing carbon and energy is transformed by the metabolic activities of heterotrophic microorganisms that bring about extensive modifications of the structural and toxicological properties of the contaminants (Joutney et al. 2013). Generally, the techniques used to biodegrade contaminants include mechanism such as abiotic oxidation, dispersion, hydrolysis, dilution, sorption, volatilization, and infiltration of nutrients containing water or other electron acceptors (Gavrilescu 2005). Most often, in situ bioremediation is applied to biodegrade the toxic waste in saturated soils and groundwater. It is a superior, cheaper, and harmless method by using microbial organisms to degrade the toxic chemicals to clean up contaminated environments. The study of in situ bioremediation chemotaxis is important because microbiota with chemotactic abilities can move into an area containing contaminants. Hence, in situ bioremediation could become a safer mechanism by enhancing the cells chemotactic abilities for degradation or transformation of hazardous compounds (Tiwari and Singh 2014). The in situ bioremediation is further divided into following categories:

#### 4.2.1.1 Bioventing

It is the technique of eliminating oxygen-sensitive contaminants from unsaturated zone by blowing air through soil. Aerobically degradable contaminants are degraded due to increased supply of oxygen in the waste, which in turn enhances the activities of native microbes. Since it is mostly aerobic process, it needs sufficient supply of oxygen or air. Sometimes, toxic soils with lower oxygen concentration are reclaimed by supplying nutrients like nitrogen and phosphorus to aid microbial biodegradation (Rockne and Reddy 2003). Oxygen is typically introduced by air injection wells that push air into the subsurface vacuum extraction wells, which draws air through the subsurface. The distribution of these nutrients and oxygen in soil is dependent on soil texture. In bioventing enough oxygen is provided through low airflow rate and amended with nutrients and moisture for microbial growth and transformation of pollutants (EPA 1997; Philp and Atlas 2005). In aerobic bioventing, air is pumped into contaminated soil above the water table through vent well. The pumped air diffuses to contaminated part of soil and removes the toxicants. The removal rate varies from one site to another site depending on soil texture and types of toxicants. However, uniform distribution of oxygen increases the rate of biodegradation. It is mainly used for the removal of hydrocarbons like gasoline, oil, petroleum, etc. (Tiwari and Singh 2014). It is more effective in high-temperature zone where water table is much below the soil surface. The shallow water table and thin soil cover are inefficient for this process of bioremediation (Rayner et al. 2007). On the other hand, very deep soil may have insufficient microbial populations for bioremediation (Frishmuth et al. 1995). In spite of these, due to simplicity, robustness, and low cost, this technique is widely adopted for bioremediation.

Sometimes, bioventing techniques are improvised into various forms depending on the types of contaminants and many other issues; few examples are cold-climate bioventing, cometabolic bioventing, anaerobic bioventing, etc. In cold-climate bioventing, soil warming is essential to speed up bioremediation process in cold-climate situations (EPA 1995), whereas cometabolic bioventing is used for bioremediation of chlorinated contaminants such as trichloroethylene, trichloroethane, and dichloroethene, which cannot be degraded by simple aerobic bioventing. This technique is based on the breakdown of a contaminant by an enzyme or cofactor that is produced during microbial metabolism of another compound (EPA 2000). The process involves the injection of electron donors, mostly volatile organic substrate such as methane, ethane, propane, butane, aromatic hydrocarbons (such as toluene and phenol), and ammonia for aerobic oxidation (EPA 2000) and methanol, glucose, acetate, lactate, sulfate, or pyruvate for anaerobic reduction (Hazen 2010). Supply of an appropriate organic substrate and air facilitates the degradation of contaminants by eliciting the production of enzymes and cofactors like monooxygenases, dioxygenase, dehydrogenase, dehalogenase, etc., which are capable of degrading the organic substrate (Karigar and Rao 2011). Like bioventing, cometabolic bioventing is equipped with similar instruments but different biological mechanism. This cometabolic bioventing technique is very useful for degrading many hydrocarbons and lightly chlorinated contaminants.

Sometimes, degradation of recalcitrant contaminants such as polycyclic aromatic hydrocarbons (PAH) and chlorinated solvents is accelerated under anaerobic conditions. This process of venting substrates other than oxygen for microbial growth is known as anaerobic bioventing (Gibbs et al. 1999; Litchfield 1993). This anaerobic bioventing also involves microbial mechanisms like fermentation and methanogenesis to destroy the contaminants. Here, in place of air, nitrogen and an electron donor are injected to create anaerobic conditions, and the nitrogen replaces oxygen from soil. Small amount of an electron donor (such as hydrogen and carbon dioxide) is produced in reducing state in the subsurface soil zone, thereby facilitating microbial dechlorination. Volatile and semi-volatile compounds may be produced, but slowly they degrade under anaerobic bioventing. These compounds can be treated in two ways. Volatile compounds may disperse into the soils surrounding the treatment zone, where aerobic degradation may occur. Halogenated and nonhalogenated semi-volatile organic compounds (SVOCs) and volatile organic compounds (VOCs) remaining in the treatment zone may be treated using aerobic bioventing followed by anaerobic bioventing. Since aerobic and anaerobic bioventing share similar gas delivery systems, the switch can be made by simply changing the injected gas.

#### **4.2.1.2 Biosparging/Air Sparging**

In biosparging, gas (usually oxygen or air) is injected below the groundwater to enhance the concentration of oxygen in the saturated zone, rather than unsaturated zone as in bioventing. It facilitates aerobic biological degradation of pollutants by naturally occurring microbiota (Singh 2006). Volatile pollutants can be eliminated from the saturated zone by air due to increased desorption and volatilization into the air stream. This technology is applied to a known source of gasoline components such as benzene, ethyl benzene, toluene, xylenes, and SVOCs in order to quantify the magnitude of remediation achieved in terms of both mass removed and decline in mass discharged into groundwater. Biosparging is most effectively used to remove light to mid-weighted petroleum-based contaminants (diesel, kerosene, and gasoline) due to their rapid volatilization (Tiwari and Singh 2014). Hence, this technique is commonly recommended for purifying aquifers and underground storage tank sites.

#### **4.2.1.3 Bioaugmentation**

It involves introduction of exogenous microorganisms having specific metabolic activity to the contaminated site for enhancing the biological degradation or transformation of organic contaminants (Suthersan 1999). Soil and groundwater contaminated with chlorinated ethane, such as trichloroethylene and tetrachloroethylene, are remedial sites where bioaugmentation is applied. The in situ microorganisms can effectively degrade these toxic contaminants to ethylene and chloride, which are

innocuous or nontoxic. However, specialized microbial population inoculated into the contaminated site may not produce desired extent of transformation of contaminants due to the influence of some biotic and abiotic factors, viz., temperature, moisture, pH, hydrolytic and osmotic pressure in environment, nutrient availability, soil type, and existing microbial composition that influences the survival and activity of microbes alone or in consortia. For bioaugmentation, microbes that have higher affinity toward hydrocarbons and ability to survive in a wide range of environment are most commonly preferred (Mrozik and Piotrowska-Seget 2010). This technique is mostly effective for degradation of light fractionated petroleum hydrocarbons ( $C_{12}$ – $C_{23}$ ) than that of heavy fractionated petroleum hydrocarbons ( $C_{23}$ – $C_{40}$ ) (Bento et al. 2005). Therefore, this method is not applicable on a wide scale for soil bioremediation.

## ***4.2.2 Ex Situ Bioremediation Approaches***

In this approach, ex situ treatments of contaminated soil or groundwater are given after excavating or pumping out of the original site. Wide range of toxic wastes and soil contaminants can be removed easily by various processes, which are categorized into slurry phase bioremediation and solid phase bioremediation.

### **4.2.2.1 Slurry Phase Bioremediation**

In this process, contaminated site is amalgamated with water and other reagents in a big tank called as bioreactor. The congenial environment in the bioreactor support optimum growth of microbes and amalgamation helps these microorganisms to remain in contact with organic toxicants present in the soil. Further, oxygen and nutrients are incorporated to enhance the breakdown rate of toxic wastes by the native microorganisms (EPA 1990). After completion of the process, water move apart from the soil, and the soil is examined and replaced into the environment. For excellent biodegradation, several biotic and abiotic factors like temperature, pH, agitation, aeration, augmentation, nutrient addition, substrate, and inoculum concentrations are the main limiting factors for biodegradation that can be manipulated easily in a bioreactor. Hence, it has greater advantages for faster bioremediation over other techniques.

### **4.2.2.2 Solid Phase Bioremediation**

In this approach, aboveground treatment of contaminated soil is done in solid phase, which can be made possible by land farming, soil bio-piling, and composting method (Girma 2015). For decontamination, excavated materials are placed in an aboveground enclosure/treatment chamber, which is facilitated with

aeration system ensuring controlled environment for optimum treatment. These methods are very easy to perform but require longer time and space, hence not commonly in use.

#### 4.2.2.3 Land Farming

Land farming is a simple approach and is also known as land treatment, in which polluted soil is excavated and spread over a prepared bed and periodically tilled to allow natural degradation of pollutants. To stimulate native biodegradative microbes and to facilitate aerobic degeneration of contaminants, a sandwich layer of excavated soil between clean soil and clay-concrete is made, where clean soil is placed at bottom and concrete layer at the top. In general, the practice is limited to treat soil of 10–35 cm depth. In land farming, various operations like tillage (for aeration), nutrient application (nitrogen, phosphorus, potassium, lime), and irrigation are performed to stimulate microbial activities for rapid bioremediation. Due to simple design, low-maintenance costs, and least cleanup liabilities, land farming is considered the best disposal alternative, mainly for the removal of pesticide residue from soil (Felsot et al. 2003). However, it is less successful for removing toxic volatiles from soil, especially in hot tropical countries.

#### 4.2.2.4 Compositing

Compositing is a biologically controlled process to treat toxic contaminants in soil by mixing materials like straw. During composting, the waste material is digested at high temperature (55–65 °C) under the influence of thermophilic microbes (NFESC 2005). During the whole degradation process, heat released in compost pit increases the temperature of material which intern leads to more solubility of waste and breakdown of complex compounds due to higher metabolic activity of microbes (Blanca et al. 2007). The soil is transported to a composting pit with a temporary structure to provide containment and protection from weather extremes. In composting, organic ingredients (agricultural wastes, vegetable wastes, sawdust and wood chips, etc.) are added with compost materials to provide supplementary carbon source. There are three designs commonly applied for composting: (1) aerated static piles (compost is formed into piles and aerated with blowers or vacuum pumps), (2) mechanically agitated in-vessel composting (compost is kept in a reactor vessel, in which it is mixed and aerated thoroughly), and (3) windrow composting (soil and organic amendments are layered in long, low, narrow piles (i.e., windrows) and periodically mixed with mobile equipment). In windrow composting, removal of the rocks and other larger particles from excavated contaminated soils becomes easier (FRTR 2003). It is a very cost-effective method and has the potential to remediate larger quantities of volatile components from contaminated soil. Composting has been successfully applicable to soils contaminated with petroleum hydrocarbons (e.g., fuels, oil, and grease), solvents, chlorophenols, herbicides, PAHs, pesticides and nitro-aromatic explosives, etc. (EPA 1998, 2004).



#### 4.2.2.5 Bio-piling

This type of ex situ bioremediation is a composite form of land farming and composting. The biopile system includes a treatment bed, an aeration system, nutrient/irrigation system, and a leachate collection system. For proper degradation there should be control of heat, moisture, oxygen, nutrients, and pH. The irrigation system is buried beneath the soil, and it provides air and nutrient through vacuum. To prevent the runoff, soil is covered with plastic to minimize evaporation and volatilization and to promote solar heating. Biopile treatment takes 20 days to 3 months to complete the degradation of petroleum hydrocarbons. It typically uses the version of land farming that helps to control physical losses of the soil contaminants by leaching and volatilization (Tiwari and Singh 2014). Other added advantage is that ambient environment for native aerobic and anaerobic microbes can easily be regulated in biopiles. Therefore, it gains popularity in comparison to the other ex situ bioremediation techniques, including land farming, in spite of its reserved space requirement.

### 4.3 Groups of Fungi Involved in Bioremediation

It is very important to understand the significance of fungal interactions and its behavior in diverse ecosystems associated to biodegradation or myco-transformation of various hazardous and toxic components. Despite an estimated 1.5 million and identified 69,000 fungal species worldwide, limited number of fungal species has been identified to be associated with mycoremediation from diverse ecology (Hawksworth 1991). The various fungal species exploited for bioremediation have been classified into various sections along with their functions and mechanisms in Table 4.1. The occurrence and growth of fungal communities depend on abiotic and biotic factors such as availability of carbon, nitrogen, phosphorus, metal ion concentration, temperature, aeration, moisture, and interspecific microbial competition. Sufficient literature exists on fungal ecology, but little information is available on the ecology of fungi associated with mycoremediation. However, based on ecology and functionality, these fungi have been grouped into following sections:

#### 4.3.1 Wood-Rotting Fungi

Wood-rotting fungi represent a diverse group of fungi associated with wood rotting. They have the ability to disintegrate wood tissue to simpler form by employing diverse enzymes to degrade complex molecules like cellulose, hemicellulose, lignin, etc. Besides wood rotting, these fungi also play significant role in bioremediation of organic pollutants. These fungal groups degrade various toxic environmental pollutants by their extracellular lignin-degrading system. Moreover, the extracellular system enables fungi to withstand considerably higher doses of hazardous

**Table 4.1** Fungal diversity exploited for mycoremediation

Groups	Fungal spp.	Used in remediation of	Mechanisms	Reference
Wood-decaying white-rot fungi	<i>Pleurotus ostreatus</i>	Cadmium	Biosorption of heavy metals	Tay et al. (2011)
	<i>Pleurotus ostreatus</i>	Biodegradable plastic	Degradation of plastic	da Luz et al. (2013)
	<i>Pleurotus sajor-caju</i>	Heavy metal, Zn	Biosorption of heavy metals	Jibran and Milsee Mol (2011)
	<i>Pleurotus tuber-regium</i>	Heavy metals	Biosorption of heavy metals	Oyetayo et al. (2012)
	<i>Pleurotus pulmonarius</i>	Crude oil	Degradation of crude oil	Olusola and Anslern (2010)
	<i>Pleurotus tuber-regium</i>	Crude oil-polluted soil	Enzymatic degradation	Isikhuemhen et al. (2003)
	<i>Bjerkandera adusta</i>	PAHs, PCBs	Enzymatic modification of lignin	Bumpus et al. (1985)
	<i>Irpex lacteus</i>	Dyes, PAHs, lindane, TNT, bisphenol A, nonylphenol, dimethyl phthalate	Enzymatic degradation [laccase, lignin peroxidase (LiP), manganese peroxidase (MnP), versatle peroxidase (VP)]	Novotny et al. (2000)
	<i>Phanerochaete chrysosporium</i>	Synthetic dyes, PAHs, lindane, polychlorophenol, DDT, PCBs	Enzymatic degradation (LiP, MnP)	Singh (2006)
	<i>Phlebia</i> sp.	PAHs, TNT, AmDNT, coal, humic acids	Enzymatic degradation (laccase, LiP, MnP)	Singh (2006)
	<i>Agaricus bisporus</i> , <i>Lactarius piperatus</i>	Cadmium (II) ions	Biosorption	Nagy et al. (2014)
	<i>Lenitula edodes</i>	2,4-dichlorophenol	Enzymatic degradation (laccase, MnP)	Tsujiyama et al. (2013)
	<i>Pleurotus platypus</i> , <i>Agaricus bisporus</i> , <i>Calocybe indica</i>	Copper, zinc, iron, cadmium, lead, nickel	Biosorption	Lamrood and Ralegankar (2013)
	<i>Pleurotus pulmonarius</i>	Radioactive cellulosic waste	Fungal mycelia solidified with Portland cement act as barrier for releasing radio-contaminants	Eskander et al. (2012)
	<i>Cortolus versicolor</i> MKACC 52492	PAH	Enzymatic degradation	Jang et al. (2009)
<i>Trametes versicolor</i>	PAHs and synthetic dyes	Enzymatic degradation	Novotny et al. (2004), Tanaka et al. (1999)	
<i>Lenitinus squarrosulus</i>	Crude oil (1–40%)	Mineralization	Adenipekun and Fasidi (2005)	

Wood-decaying brown-rot fungi	<i>Fomes fasciatus</i>	Copper (II)	Biosorption	Sutherland and Venkobachar (2013)
	<i>Daedalea dickinsii</i> , <i>Fomitopsis pinicola</i> , <i>Gloeophyllum trabeum</i>	DDT	Microbial biodegradation via Fenton reaction	Purnomo et al. (2011)
	<i>Flammulina velutipes</i>	Copper	Biosorption	Luo et al. (2013)
	<i>Fomitopsis palustris</i>	Metals (Zn, Co, Cu)	Oxalate production	Gadd et al. (2014)
	<i>Postia placenta</i> , <i>Gloeophyllum trabeum</i>	Polysaccharide decomposition	Lignin demethylation	Filley et al. (2002)
	<i>Gloeophyllum striatum</i>	Degradation of polychlorophenol	Mineralization	Singh (2006)
	<i>Schizophyllum commune</i> , <i>Polyporus</i> sp.	Malachite green dye	Enzymatic degradation	Rajput et al. (2011)
	<i>Agrocybe praecox</i>	PAHs, TNT	Modification by laccase and MnP	Steffen et al. (2000)
	<i>Nematoloma frowardii</i>	Radionuclide C	Enzymatic degradation (MnP)	Hofrichter et al. (1999)
	<i>Stropharia coronilla</i>	Mineralization of <sup>14</sup> C-labeled synthetic lignin	Degradation by Ligninolytic enzymes	Singh (2006)
Soil fungi	<i>Mucor</i> sp.	Heavy metals (Ni, Cd, Pb, Zn)	Bioadsorption of heavy metals	Yan and Viraraghavan (2000)
	<i>Rhizopus</i> sp.	Heavy metals (Cd, Cu, Zn)	Biosorption of heavy metals	Volesky and Holan (1995)
	<i>Cunninghamella</i> sp.	Heavy metals (Pb, Cu, Zn)	Ions-sequestration	El-Morsy (2004)
	<i>Mortierella</i> sp.	2,4-D (2,4-dichlorophenoxy acetic acid)	Hydroxylation and dechlorination	Nakagawa et al. (2006)
	<i>Mortierella</i> sp. Gr4	Phenylurea herbicides, viz., chlorotoluron, diuron, linuron, isoproturon	N-demethylation and hydroxylation	Badawi et al. (2009)
	<i>Aspergillus niger</i>	Heavy metals (Cd, Zn, U, Ag, Cu)	Biosorption of heavy metals	Wang and Chen (2006)
	<i>Aspergillus fumigatus</i>	Heavy metal (U)	Bioaccumulation of heavy metal	Guibal et al. (1995)
	<i>Trichoderma viride</i> , <i>Humicola insolens</i>	Heavy metal (Hg)	Biosorption of heavy metal	Muhammad et al. (2007)
	<i>Paeclomyces</i> sp., <i>Penicillium</i> sp., <i>Phlebia</i> sp.	PAH, Endosulfan	Hydroxylation	Anastasi et al. (2009), Tigrini et al. (2009)

(continued)

**Table 4.1** (continued)

Groups	Fungal spp.	Used in remediation of	Mechanisms	Reference	
Mycorrhizal fungi	<i>Glomus geosporum</i>	Zn	Enzymatic degradation	Leyval et al. (1997)	
	<i>Stiellus granulatus</i>	Cresol, catechol	Biotransformation	Singh (2006)	
	<i>Scutellospora heterogama</i>	Cu	Enzymatic degradation	Sambandan et al. (1992)	
	<i>Gigaspora gigantea</i>	Zn, Cu, Pb, Ni, Cd	Enzymatic degradation	Sambandan et al. (1992)	
	<i>Rhizogonon vinicolor</i>	2,4-D	Mineralization	Leyval et al. (1997)	
	<i>Hymenoscyphus ericae, Oidiodendron griseum</i>	2,4-D, atrazine	Mineralization via enzymatic degradation	Donnelly and Fletcher (1994)	
	Endophytic fungi	<i>Cortiolopsis gallica</i>	PAH	Biotransformation	Pickard et al. (1999)
		<i>Ceratobasidium stevensii</i>	Phenanthrene	Enzymatic degradation (MnP)	Dai et al. (2010)
		<i>Phanerochaete chrysosporium</i>	PAH degradation	Enzymatic degradation (LiP, MnP)	Dhawale et al. (1992)
		<i>Bjerkandera</i> sp.	Phenanthrene	Oxidation	Terrazas et al. (2005)
<i>Phomopsis</i> sp.		PAH degradation	Enzymatic degradation	Tian et al. (2007)	
<i>Nia vibrossa, Julella avicimae, Lignicola laevis</i>		Polymeric dyes	Enzymatic degradation	Pointing et al. (1998)	
Aquatic fungi	<i>Aspergillus sclerotiorum</i> CBMAI 849, <i>Cladosporium cladosporioides</i> CBMAI 857, <i>Mucor racemosus</i> CBMAI 847	Lignin-based industrial pollutant	Mineralization	Raghukumar et al. (1996)	
	<i>Phaeosphaeria spartanicola, Halosarphaeia rainagiriensis, Sordaria fimicola</i>	Industrial pollutant	Mineralization	Bonugli-Santos et al. (2010)	
	<i>Penicillium raistrickii</i> CBMAI 931, <i>Aspergillus sydowii</i> CBMAI 1241, <i>Trichoderma</i> sp.	Profenofos	Enzymatic degradation	da Silva et al. (2013)	

pollutants (McErlen et al. 2006). The added advantage of utilizing these fungi is that they are nonspecific and nonselective in biodegradation. Thus, they do not need any preconditioning prior to transformation of pollutants (Azadpour et al. 1997). Hence, they have wider adaptability and extensive degradative ability. Wood-degrading fungal species significantly differ in their colonization ability and can be characterized as strong competitors (i.e., *Pleurotus* spp., *Phanerochaete* spp., *T. versicolor*) and weak competitors (*Dichomitus squalens* and *Ganoderma applanatum*) (Baldrian 2008). Based on the mode of attack on the woody tissue, these fungi are categorized as white-rot fungi and brown-rot fungi.

#### 4.3.1.1 White-Rot Fungi

Among diverse wood-rotting fungi, only certain fungi have the unique ability to degrade lignin along with cellulose and hemicellulose and result in white bleaching of woods during decay, hence, considered as white-rot fungi. They mainly include fungi of *Basidiomycota*, and few belong to *Ascomycota*, exclusively members of *Xylariaceae* family. These white-rot fungi were the first to be used to study mycoremediation. They produce some enzymes like lignin peroxidase, manganese peroxidase,  $H_2O_2$ -generating enzymes, and laccase. Among them laccase initiates ring cleavage, and others generate strong oxidants, which result in the disintegration of lignin/xenobiotic molecules (Anastasi et al. 2009; Kirk and Farrell 1987). The biodegradation of complex molecules by extracellular oxidative ligninolytic enzymes has been studied in detail in *Phanerochaete chrysosporium*. Based on this, Bumpus et al. (1985) proposed use of this fungus in bioremediation studies, and this has emerged as an archetypal model system for mycoremediation. *P. chrysosporium* has the ability to degrade toxic or insoluble compounds to  $CO_2$  and  $H_2O$  more efficiently than other fungi or microbes. The diverse oxidative and reductive methods of degradation or biotransformation of recalcitrant compounds make its application magnetic in various matrices. It can be used effectively to remove broad spectrum of aromatic compounds and xenobiotics present in contaminated soil due to nonspecific and robust nature of ligninolytic enzymes (Schauer and Borriss 2004). In addition to *P. chrysosporium*, several other white-rot fungi (e.g., *Pleurotus ostreatus*, *Trametes versicolor*, *Bjerkandera adusta*, *Lentinula edodes*, and *Irpex lacteus*) are also known to degrade these compounds (Singh 2006). Past two decades of literature indicates that the white-rot fungi account for at least 30% of the total research on mycoremediation (Borràs et al. 2010; Singh 2006). It indicates the immense potentiality of this group of fungi in mycoremediation of environmental pollutants and toxicants from soil.

#### 4.3.1.2 Brown-Rot Fungi

This group of wood-rotting fungi is responsible for the degradation of cellulose and hemicellulose present in wood, leaving lignin more or less intact as a brown chemically modified component. Hence, they are known as brown-rot fungi. They mainly

belong to the phylum *Basidiomycota* under class *Agaricomycetes*. Majority of the brown-rot fungi are distributed in *Agaricales*, *Hymenochaetales*, *Gloeophyllales*, and *Polyporales*. They partly modify the lignin via demethylation, partial oxidation, and depolymerization by a nonenzymatic Fenton-type catalytic system. The partially modified lignin gives distinctive dark brown to the decayed wood. Interestingly, only 6% of all the known wood-decay fungi are recognized to cause a brown rot and are preferably associated with conifers (Anastasi et al. 2009). The brown-rot fungi degrade cellulose and hemicellulose by an oxidative process, involving the production of hydrogen peroxide, which helps in the synthesis of free hydroxyl (OH) radicals and that in turn facilitate the degradation and mineralization of synthetic chemotherapeutants. In addition to this, antimicrobial drug tolerance and oxalic acid production increase their metal degrading ability. This potentiality of brown-rot fungi can be exploited in larger scale for bioremediation.

### 4.3.2 Leaf-Decomposing Fungi

Leaf-decomposing fungi are one of the main components of forest ecology. They actively take part in the decomposition of wood and litter, humification, and mineralization of soil organic matter. Fungal community undergoes rapid successional changes during leaf litter decomposition (Singh 2006). Fungi of *Ascomycota* phylum are predominant during the initial stages of litter decay, but their population gradually decreases with increase in fungi of the *Basidiomycota* phylum during the later stages of decomposition (Osono 2007). Both these fungal group play a vital role in the decomposition of lignocellulolytic materials of plant litters. Basidiomycetous litter fungi produce numerous ligninolytic enzymes like cellulase, laccase, and oxidoreductases that are essential for degradation of plant materials deposited on forest floor (Osono and Takeda 2002). Their enzymes also take part in degradation of organic pollutants, pesticides, and herbicides that are persistent in soil (Aranda et al. 2010; Casieri et al. 2010; Farnet et al. 2009). Therefore, utilization of these fungi will open up new scope for bioremediation of agricultural soil.

### 4.3.3 Soil Fungi

Soil fungi represent heterogeneous groups, especially *Ascomycota*, *Chytridiomycota*, and *Zygomycota*. They are important components of soil ecology and play a crucial role in organic matter decomposition and carbon and nitrogen cycling in soil. They are saprophytes and have very good cellulose-decomposing ability but are mostly non-ligninolytic in nature. They mostly include different species of genera *Acremonium*, *Allescheriella*, *Alternaria*, *Aspergillus*, *Beauveria*, *Cladosporium*, *Cunninghamella*, *Engyodontium*, *Fusarium*, *Geomyces*, *Microsporium*, *Mortierella*, *Paecilomyces*, *Penicillium*, *Phlebia*, *Rhizopus*, *Stachybotrys*, and *Trichoderma*

(Anastasi et al. 2009; D'Annibale et al. 2006; Pinedo-Rivilla et al. 2009; Tigini et al. 2009). The extracellular enzymes like monoxygenase produced by non-ligninolytic fungi lead to degradation of PAHs via hydroxylation. They are also tolerant to pollutants such as PAHs, polychlorobenzoic acids (PCBs), chlorobenzoic acids (CBA), and endosulfan, which indicate their potentiality as bioremediation agents in soil (Garon et al. 2000; Tigini et al. 2009; Pinedo-Rivilla et al. 2009). The fungi degrading recalcitrant polymers are often predominant in the later stages of decomposition. The ecological succession of these fungi later in the decomposition sequence is related to their specialized ability to degrade complex polymers such as lignin and keratin that most of the other fungi cannot utilize. Thus, they are often considered as very good xenobiotics-degrading fungi, and their consortia with different species ensure a greater effectiveness in soil bioremediation.

#### **4.3.4 Mycorrhizal Fungi**

Mycorrhizae fungi have symbiotic association with plant roots and establish mutualistic relationship by facilitating nitrogen and phosphorus supply to the plants and in turn derive organic carbon from plants for fungal metabolism (Goltapeh et al. 2008; Prasad et al. 2005, 2017). There are several types of plant mycorrhizal associations, viz., ectomycorrhiza, ectendomycorrhiza, ericoid mycorrhiza, arbuscular mycorrhiza, arbutoid mycorrhiza, monotropoid mycorrhiza, and orchid mycorrhiza. They are mainly involved in nutrient supply to plant and provide protection against various environmental stresses including water stress, metal toxicity, etc. They can ameliorate heavy metal toxicity by reducing metal translocation within plant system (Leyval et al. 1997; Kamal et al. 2010); thereby, they help plants to adapt and survive in heavy metal-polluted sites and degraded lands like coal mines and waste sites (Gaur and Adholeya 2004). On the other side, the host plant accommodates selective advantage to fungus for establishing at a contaminated site and metabolizing various polycyclic aromatic hydrocarbons, petroleum, and chlorinated aromatic pesticides, such as 2,4-dichlorophenoxyacetic acid (2,4-D) and atrazine via enzymatic degradation (Donnelly and Fletcher 1994). Thus, it will be very noteworthy to decipher the role of mycorrhizal fungi in the bioremediation of hazardous compounds in soil.

#### **4.3.5 Endophytic Fungi**

Plant endophytes are the microbial group including fungi and bacteria that have colonizing ability within plants without causing any negative impact. They mostly reside inside specific plant tissues like root cortex, vascular bundle, apoplastic space, young buds, and even in dead bark cell and perform diverse metabolic activity for methane assimilation, nitrogen fixation, etc. Several of them have

saprotrophic ability to sustain in the dead litter (Osono 2006). They are equipped with an array of enzymes like cellulase, lipase, peroxidase, and protease (Orlandelli et al. 2015; Petrini et al. 1992) for bioremediation of environmental pollutants (pesticides, herbicides, insecticides, petrochemicals, polychlorobiphenyls, polyaromatic hydrocarbons, polyester polyurethane) and biotransformation of heavy metals (Dai et al. 2010; Russell et al. 2011). In this way, they enhance plant tolerance and adaptability to contaminant and metal toxicity. Thus, they can be employed as valuable tools for bioremediation.

### 4.3.6 Aquatic Fungi

Aquatic environment is very complex, and it supports a wide range of activities that influence the microbial life. Microbial communities are important ecological components in aquatic environments, and among them, aquatic fungi are one of the most predominant groups, essential for various biogeochemical processes. They can be either obligate or facultative in nature and are distributed from freshwater to marine ecosystem. They produce diverse enzymes like laccase, lignin peroxidase, and Mn-peroxidase that are essential for decomposition of lingo-cellulolytic materials as well as lignin-based industrial pollutants. It ensures wider adaptability of these fungi in extreme environment like high salinity, high pressure, low temperature, metal, and oil spill toxicity. This can be evident from the production of ligninolytic enzyme and subsequent mineralization of lignin-based compound to CO<sub>2</sub> by many aquatic fungi like *Aspergillus sclerotiorum* CBMAI 849, *Cladosporium cladosporioides* CBMAI 857, *Mucor racemosus* CBMAI 847, *Phaeosphaeria spartinicola*, *Halosarphaea ratnagiriensis*, *Sordaria fimicola*, and *Flavodon flavus* (Bonugli-Santos et al. 2010; Raghukumar et al. 1996). Similarly, some marine aquatic fungi like *Penicillium raistrickii* CBMAI 931, *Aspergillus sydowii* CBMAI 935, *Aspergillus sydowii* CBMAI 1241, and *Trichoderma* sp. CBMAI 932 have been tested for their biocatalytic potential to degrade profenofos (da Silva et al. 2013). Thus, aquatic fungi are said to possess the potentiality that would open up the scope for mycoremediation of pesticides and industrial effluents.

## 4.4 Mechanisms of Mycoremediation

Microbes are ubiquitous and have wider distribution in various environmental *milieus*. Among various microorganisms, fungi are very opportunistic and have wide adaptability and quick responsiveness to stress condition, environmental disasters, and extreme climatic situations. They can degrade complex hydrocarbons and chains of hazardous molecules into simpler, nontoxic, biodegradable form to clean up the environment. Many fungi also have excellent capacity to bind with metal ions, which includes the efflux of metal ions outside the cell and



accumulation and formation of metal ion complex inside the cell, and later they reduce the toxic metal ions to a nontoxic state. Several mechanisms have evolved by which they can immobilize, mobilize, or transform metals rendering them inactive or tolerate the uptake of heavy metal ions. The mechanism adopted by fungi for bioremediation include:

- (i) exclusion—the metal ions are kept away from the target sites by formation of a permeable barrier
- (ii) extrusion—the metals are pushed out of cells by active transport
- (iii) fixation—fix metals by forming complex with metal-binding proteins or other cell components like enzymatic detoxification, intra- and extracellular sequestration, dissolution of metal by acid production, chelation, and precipitation through the production of organic bases, extracellular metal precipitation
- (iv) biotransformation—toxic metal is reduced to less toxic forms like methylation, demethylation, volatilization, oxidation, and reduction

In general, immobilization, mobilization, biosorption, and biotransformation are considered main approaches used for mycoremediation of hazardous in the agroecosystem in order to avail good air and water quality for future generations.

#### ***4.4.1 Immobilization***

Microorganisms utilize this approach to alter physical or chemical characteristics of contaminants for reduction of its mobility. This can be accomplished by physically restricting contact between the contaminants or by chemically altering the contaminants. For immobilization of the toxic contaminants, most of the contaminated sites use solidification and stabilization approach. This approach emphasizes mixing of the contaminated material with appropriate amounts of water and stabilizer. The mixture results in the formation of a solidified matrix with the toxic waste. Heavy metals can be precipitated by injecting chemicals to the contaminated soil, which leads to formation of metal hydroxides. The chemical composition of the site, the amount of water present, and temperature are the key factors for the successful use of this mechanism (Gadd 2004). The stabilization and solidification techniques can occur both in situ or ex situ. However, in situ method is preferred for volatile or semi-volatile organics and for treatment of surface or shallow contamination of soil.

#### ***4.4.2 Mobilization***

Microorganisms can mobilize contaminants through leaching, chelation by microbial metabolites and siderophores, alkylation, methylation, and redox transformations. Leaching occurs when acidification of soil environment takes place through microorganisms by their proton efflux leading to acidification and resulting in

release of free metal cations. In most fungi, leaching is generally mediated through the production of low-molecular-weight organic acids, which upon breakdown provide protons and metal-complexing organic acid anions. *Trichoderma harzianum* is regarded to have the potentiality to solubilize  $MnO_2$ ,  $Fe_2O_3$ , metallic zinc, and rock phosphate through the mechanism of chelation and reduction (Altomare et al. 1999).

Siderophores are low-molecular-weight iron-chelating legends, which are able to bind to other metals such as magnesium, manganese, chromium, gallium, and radionuclide (e.g., plutonium). Alkylation involves the transfer of an alkyl group from one molecule to another, which can be transferred as an alkyl carbocation, a free radical, a carbanion, or a carbene. Methylation involves incorporation of methyl groups that are enzymatically transferred to a metal, forming a number of different metalloids. Redox transformations can allow microorganisms to mobilize metals, metalloids, and organometallic compounds by reduction and/or oxidation processes. In addition, various metal-mobilization techniques can also occur in nature.

#### 4.4.3 Biosorption

Biosorption is a physicochemical approach which involves uptake of toxicants from dead/inactive biological sources using mechanisms like adsorption, chelation, precipitation, reduction, ion exchange, and coordination with suitable functional groups (amine, hydroxyl, carboxyl, phosphate, and sulfhydryl) present on cell surface (Strandberg et al. 1981). Biosorption process can be rapid, reversible, and unique and should encourage sequestration of dissolved metals from very dilute and complex solutions efficiently. The process of biosorption involves a solid phase (biosorbent) and a liquid phase (solvent) containing dissolved material, which is to be sorbed (Dhankhar and Hooda 2011). Fungal biomass receives much attention due to the high percentage of cell wall materials which act as biosorbents. It increases the variety of functional groups involved in metal binding and their sequestration by fungi like *Mortierella ramannianc*, *Rhizopus sexualis*, *R. stolonifer*, *Zygorhynchus heterogamus*, *Z. moelleri*, *Aspergillus niger*, *Mucor racemosus*, *Penicillium chrysogenum*, and *Trichoderma viride* (Azab et al. 1990; Kurek et al. 1982; Ross and Townsley 1986). Thus, biosorption contributes in complementary manner to the overall sequestration of toxic pollutant even from very small concentrations.

#### 4.4.4 Biotransformation

Biotransformation of metal/metalloids and radionuclide can be done by exploiting the microorganisms that can modify the microenvironment near the microbial cell through catalysis, oxidation, and reduction of the solubility/mobility of metal through methylation and/or demethylation (Singh 2006). The microorganisms contribute possible physicochemical mechanism of interaction with metals or metal ions together with other metabolically mediated mechanisms such as bioprecipitation and bioreduction.

#### 4.4.4.1 Bioprecipitation

It helps in modification of the environment through metabolic mediated processes around the microbial cell. Under aerobic conditions, microbes grow by the transfer of electrons available from the electron donor molecule to the oxygen (Remoudaki et al. 2007). Mineralization of organic carbon into carbon dioxide and reduction of oxygen into water increase the alkalinity and pH of the cell microenvironment, and the excess bicarbonate formed favors the precipitation of metal ions as metal hydroxides  $Me(OH)_x$  or carbonate  $Me_2(CO_3)_x$ .

#### 4.4.4.2 Biological Oxidation/Reduction

Reduction of heavy metals such as Fe(III) to Fe(II), Mn(VI) to Mn(II), Cr(VI) to Cr(III), Se(VI) to Se(IV), As(V) to As(III), Mo(VI) to Mo(IV), and U(VI) to U(IV) can be catalyzed by the enzymes from a wide variety of microorganisms. These reduced elements serve as electron acceptors in alternative microbial respiration or reduced by the enzymes without energy production (Gadd 2004).

Mechanisms of immobilization, mobilization, biosorption, and biotransformation of metals/metalloids, radionuclides, and related organic substances have potential for remediation of contaminated sites with the use of native microorganisms especially fungal biomass.

### 4.5 Application of Mycoremediation

Mycoremediation is a form of bioremediation in which native fungi are applied to surface soils to remove and degrade toxic contaminants. It involves different practices like bioaugmentation, biostimulation, biotransformation, biosorption, etc. which have several important applications for the management of industrial wastewaters, petroleum hydrocarbons, heavy metals, distillery and brewery wastes, dyes, pesticides, pulp and paper mill effluent, bleach plant effluent, wood preservatives, organochlorines, and many other hazardous contaminants (Ma and Zhai 2012).

#### 4.5.1 Mycoremediation of Soil

Mycoremediation (fungal-based technique) of soil can be accomplished using several strategies depending on site, conditions, and typically involved chemical pollutant. Fungi have also demonstrated the adsorption of heavy metals, degradation and mineralization of phenol/phenolic compounds, petroleum hydrocarbons, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, chlorinated insecticides and pesticides, dyes, biopolymers, and other substances in various matrices with

suitable mechanisms. Similarly, fungi have the capacity to break down and disassemble long-chain toxins into simpler and less toxic chemicals. Keeping this in view, role of fungi in mycoremediation for treatment or biodegradation of pesticide residue, heavy metals, and xenobiotic compounds for bioprospection of agricultural soil in sustainable development is being discussed in this section.

#### 4.5.1.1 Biodegradation of Pesticide Residue

Depending upon usage, pesticides are of different types—insecticide, fungicides, nematicides, and weedicides, which are used, respectively, against various pests like insects, fungi, nematodes, and weeds. These chemicals are used frequently and intensively, which lead to the persistence of these harmful pesticides in soil. In nature, the pesticide residues are degraded through physical, chemical, and biochemical means, but because of their high stability and water solubility, the residues persist in the soil as well as environment for longer time. There have been instances of pesticide persistence and their unwanted side effects on the various components of the agroecosystems, thereby bringing risk to the major forms of life (Gurug and Tanabe 2001; Meriel 2005; Sivasankaran et al. 2007). The fungal degradation of pesticides is carried out by lignin-degrading enzymes or by other enzymatic systems in mycoremediation. Oxidoreductases, laccase, and peroxidases are the fungal enzymes which have prominent application in the removal of polyaromatic hydrocarbons (PAHs) contaminants from freshwater, marine water, or terrestrial sites. Among the white-rot fungi group, *Phanerochaete chrysosporium*, *Agrocybe semi-orbicularis*, *Auricularia auricula*, *Coriolus versicolor*, *Dichomitus squalens*, *Pleurotus ostreatus*, and *Flammulina velutipes* degrade a wide variety of pesticides such as aldrin, atrazine, diuron, terbuthylazine, dichlorodiphenyltrichloroethane (DDT), gamma-hexachlorocyclohexane, dieldrin, metalaxyl, heptachlor, chlordane, lindane, mirex, etc. (Odukkathil and Vasudevan 2013). For the degradation of persistent chemical substances/pesticides, involvement of enzymatic reactions has been observed to have high bioremediation potential, and it represents one of the most important strategies for the degradation of hazardous. Hence, fungal bioremediation is one of the most promising approaches to overcome the pesticide problem of cultivable soils.

#### 4.5.1.2 Bioremediation of Heavy Metals

Heavy metal pollution of soil is one of the most serious concerns in the agroecosystem. Industrial effluents and disposal of sewage and concentrated metal wastes are mainly responsible for the accumulation of these heavy metals in soil, which has negative impact on yield as well as crop quality (Singh and Gauba 2014). Fungi are cosmopolitan in nature and play a promising role in natural remediation of heavy metals from agricultural land by channeling them to the fungal fruiting bodies (Dugal and Gangawane 2012). Decomposition of hazardous substances in the

ecosystem is one of the primary roles of fungi, performed by its mycelium, which secretes extracellular enzymes and acids that break down the toxic contaminants. Fungal biomass has a high percentage of cell wall material that shows excellent metal-binding properties. Their heavy metal-binding capacity is dependent on the mycelial age and on the culture media composition used for cultivation. Similarly, fungal cell wall also plays a key role in heavy metals sorption, as isolated cell wall fraction contributes about 38–77% of metal uptake and its sorption capacity is 20–50% higher than the overall binding capacity of the mycelium. Fungi are a versatile biosorption group as they can grow under extreme conditions of pH, temperature, and nutrient availability as well as high metal concentration. Mushrooms are also excellent in heavy metal biotransformation and are considered good recyclers. They can be found in all sorts of environment, as they are capable to grow on logs, animal dung, agricultural wastes, lawns, etc. (Demirbas 2001). However, for mycoremediation of heavy metals, it is important to determine the right fungal species to target a specific heavy metal toxicant because they should be able to absorb the soluble substances so formed. Therefore, there is a need of extensive exploration and bioprospection for fungal species for removal of heavy metals in the field, because mycoremediation is a potential tool which can expedite the process of sequestration or degradation of contaminants like heavy metals from soil.

#### 4.5.1.3 Degradation of Xenobiotics

Accumulation of recalcitrant xenobiotics is one of the major concerns of high input-based agricultural systems. Major xenobiotics include alkanes, polycyclic hydrocarbons (PAHs), synthetic azo dyes, fuels, solvents, antibiotics, pollutants (dioxins and polychlorinated biphenyls), chlorinated, polyaromatic, and nitro-aromatic compounds (Sinha et al. 2009). They are thermodynamically stable, can persist (via micropore entrapment and soil accumulation) in the environment for several decades, as well as pose toxicity to soil health, public health, and environmental health. Therefore, physicochemical and biological methods have been employed for the biodegradation of xenobiotics. Physicochemical methods are expensive, whereas biological methods include microorganisms (fungi, aerobic and anaerobic bacteria) and are comparatively safer to the environment. This method of biodegradation has high ecological and eco-friendly significance as it depends on the indigenous microbial diversity participating in mineralization of hazardous contaminants and their transformation or immobilization into less harmful or nonhazardous compounds. In degradation of such compounds, enzymes like oxygenase play major role by breaking down the ester, amide, or ether bonds and in some cases the aliphatic chains and cyclic rings in aromatic compounds. However, presence of a suitable site is required for accurate action of this enzyme. In addition, its concentration and favorable reaction conditions are required for induction of breakdown of such compounds. Sometimes, xenobiotic compounds are often utilized as a source of energy, nitrogen, carbon, or sulfur by various microorganisms. *Aspergillus* and *Penicillium* are one of the most predominant organisms in soil that show high oil degrading

capability (Hamsavathani et al. 2015). The degradation of xenobiotic compound can take place through white-rot fungi with certain enzymes, whereas degradation of trinitrotoluene (TNT) has been reported by non-ligninolytic strains of *P. chrysosporium* (Singh 2006). Bioremediation is one of the useful cleanup processes that exploit the catabolic abilities of microorganisms to degrade harmful and toxic xenobiotics. Moreover, it maximizes the potential benefits of microbial community in combating pollution problems from xenobiotic compounds.

### 4.5.2 Mycofiltration of Water

Mycofiltration is the pioneering technique of using fungi to filter out pollutants from agricultural runoff, estuaries, storm water, and gray water. This technique was first developed in the USA by Paul Stamets in the late 1980s. Mycelium is the vegetative structures of fungi which are able to hold soils together, absorb nutrients, and, in certain species, consume harmful bacteria. Fungi perfecti is identified as a creator of an innovative, low-cost bacterial removal system called mycofiltration—using mycelium to treat contaminated storm water runoff. For example, the mycelium of *Stropharia rugosoannulata* (“garden giant” mushroom) can survive under harsh environmental stresses and remove large amounts of *E. coli* from flowing water. Use of fungal biomass to filter water is one of the most efficient applications of mycoremediation, where the network of fungal cells produces a living micron filter to trap or inactivate the pathogens physically as well as biologically (Tiwari and Singh 2014). Chemical degradation in water can be accomplished using enzyme harvesting systems and in-line filters of biomass to conclude the extent of contact time required to achieve a needed safety level to release the output into the environment (Bernasconi et al. 2006). Living swales and rain gardens lined with mycelium offer this type of biological and chemical activity. It should be designed on the basis of trial to determine the amount of biomass and fungal species needed for most effective application of bioremediation. The mycofiltration research should be blended with the application of fungal biotechnology, which provides an innovative and interdisciplinary knowledge for remediation of contaminants from water sources.

Agricultural industry is reported to produce approximately 40 billion metric tons of organic wastes worldwide, which involve wastes produced by human, livestock, fishery, forestry, and crop residues; so its disposal has become a prime global priority in eco-friendly manner (Suthar 2007). Therefore, much attention is being paid recently to develop low-input, effective, and efficient technologies to convert such nutrient-rich organic wastes into value-added products for sustainable land uses. However, these can be managed through composting (through the action of lignocellulolytic microorganisms) and vermicomposting (joint action between the earthworms and microbes where microbes help in degradation of organic matter and earthworms drive the process, condition the substrate, and alter the biological activity) (Gaur 1999). Composting of agricultural residues becomes easier with the help

of several fungi like *Trichoderma harzianum*, *P. ostreatus*, *Polyporus ostriformis*, and *P. chrysosporium*. These recycle the lignocellulosic waste with high economic efficiency (Singh and Nain 2014). The recycled material plays important role when applied to soil and improves soil fertility and health. Hence, bioconversion of agro-wastes may be utilized as a useful resource for production of food, feed, biofuels, and sustainability of the agroecosystems.

## 4.6 Conclusion and Future Prospects

Advancement in modern agriculture has made tremendous progress in supply of food to the growing populations. However, increased population, industrialization, and urbanization are responsible for environmental contamination and degradation. These contaminants in agriculture are divided into four main groups: agriculture and allied waste, pesticides and its residues, fossil fuel and its combustion products, and heavy metals and xenobiotics. Fertilizers and agro-waste are subjected to the common recycling processes constantly operating in nature, whereas fuel, pesticide, and heavy metal pollution are often long-term and cause persistence problems due to their recalcitrance, xenobiosis, and potential toxicity. Degradation of such pollutants can be done by chemical, physical, or biological means. However, biological methods are performed due to low-cost and minimal adverse effect on the environment. The biological approach of remediating hydrocarbon, organic wastes, and heavy metals with the help of fungal agents to reclaim contaminated soils and water resource is a necessity in order to have a safe and sustainable environment. Fungi or fungal mycelia are among the major decomposers and play major role as natural remediators. They have the ability of enzymatic degradation and mineralization, release and store various elements/ions, and accumulate toxic materials, which facilitate energy exchange between the aboveground and belowground systems. It has proven the modification of soil permeability, ion exchange, and detoxification of contaminated soil. It has become an economically and environmentally attractive alternative, as it does not leave behind products that are comparatively more toxic to the environment than the substrates.

The knowledge of methodologies and ecological tools to sustain fungal biomass and their activity and enzymatic production into contaminated matrices is still limited and is considered as a great hindrance in the process of mycoremediation. Further, greater emphasis is essential for the application of this technology on large-scale projects, which demand advanced research along with integration of biotechnological, microbiological, and engineering tools to streamline the methodologies to achieve enhanced success of mycoremediation. Although endophytes and aquatic fungi play an indispensable role in mycoremediation, limited findings are available regarding nutritional profiles and enzyme activities of aquatic fungi and endophytes. Greater attempts are needed to employ aquatic fungi and endophytes in the mycoremediation of contaminated water and soil resources. Extensive research is needed to develop application methods of

mycofiltration and degradation of xenobiotics and agricultural waste that may turn more reliable and helpful in the understanding of degradation mechanisms as well as physiological and enzymatic regulators involved in the mineralization of contaminants particularly for remediation of agricultural soils.

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