Chapter 5
Phytoremediation and Fungi: An Underexplored Binomial

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Contents

5.1 Environmental Pollution: A General Background

Environmental pollution is a worldwide issue that should concern governments and society since it is an ecological problem and a threat to human health. Pollution is defined as the introduction of contaminants into the air, water, and soil that can cause damage to different organisms whose origins are mainly caused by human activities. Some of these activities include refining and distribution of fuel from fossil oil; gas exhaustion from automobiles; domestic, industrial, and agricultural activities; and erroneous deposition of pharmaceutics. The chemicals derived from these activities are often a threat to life and are referred to as xenobiotics (Godheja et al. 2016).

The word "xenobiotic" is derived from two Greek words: *xeno* and *biotic*. In English, the first means strange, unnatural, or different, while the second refers to life. Xenobiotics, also called organic micropollutants, are those organic chemicals

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that are not present in the biosphere prior to artificial synthesis or not present in high concentrations without human activities, to which an organism is exposed but which are extrinsic or foreign to its normal metabolism (Sánchez-Avila and Kretzschmar 2017).

Xenobiotics have been classified as classics and emergents. Among the classics are polyaromatic hydrocarbons, cyclic biphenyls, nitroaromatic compounds, aliphatic and aromatic halogenated compounds, triazines, azo dyes, and organic sulfonic acid (Godheja et al. 2016), while examples of emergents are pharmaceutical drugs and their residues, such as analgesics and nonsteroidal antiinflammatory drugs (NSAIDs) like ibuprofen and paracetamol (Joanna et al. 2018). Classic xenobiotics are those whose presence can cause adverse effects, which have been reported for several years, while emergents are those whose toxicity or effects have not been fully comprehended, despite their wide prevalence (Joanna et al. 2018).

Since 1939, with the discovery of the pesticide DDT (dichlorodiphenyltrichloroethane) by chemist Paul Herman Mueller, xenobiotics have been present in the environment. Since 1939, with the discovery of the pesticide DDT (dichlorodiphenyltrichloroethane) by chemist Paul Herman Mueller, xenobiotics have been present in the environment and, more than 600 chemical pesticides have been probed and registered in the USA. Xenobiotics have been reported to cause water pollution, and those that are persistent contribute to soil contamination.

The aquatic enviroments, such as lakes, rivers, seas and groundwater, are strongly affected by organic micropollutants, which are present at nanogram to milligram per liter and can have a negative impact in aquatic organisms (De los Ríos et al. 2016). It has been reported that there are trace amounts of xenobiotics in aquatic environments, such as alkylphenols, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls, and phthalate esters (Sánchez-Avila et al. 2012; Gao and Wen 2016), among others.

Since xenobiotics are toxic and their rate of degradation is very slow, adverse effects on human and ecological health are possible. Therefore, xenobiotic concentrations in the environment should be maintained at as low level as possible. In consequence, remediation technologies are needed to remove them partially or completely from nature.

5.2 Remediation Technologies

The contamination of soil, groundwater, and surface water due to pollutants derived from human activities involving agriculture, mining, industrialization, petroleum extraction, and transport is a global health problem that causes the death of thousands of people around the world. It also causes large ecosystem losses and is linked to global climate change. Almost all human activities produce xenobiotics, which, if not properly treated, can reach noncontaminated areas and extend their deleterious effects. Since 1950, more than 140,000 chemicals and pesticides have been synthetized, and 2500 of them are widely dispersed in the planet and have never been tested for toxicity (Landrigan et al. 2017). Nations have been implementing policies to reduce the production and dispersion of contaminants and have been developing technologies to control pollution. However, some other problems need to be addressed, such as safely removing or destroying the contaminants that have already been produced and dispersed. In the USA, there are 350,000 contaminated sites in need to be cleaned up over the next 30 years. In Europe, there are 3,000,000 potential contaminated sites, of which >8% (~250,000 sites) are highly contaminated (Gong et al. 2016).

Contaminated sites can have prompted the development of remediation technologies, which have been evolving during the last 30 years. The main contaminants of soils and water are heavy metals, petroleum-derived hydrocarbons, aromatic compounds, pesticides, and fertilizers. Different technologies have been created to remove these noxious substances and/or convert them into less toxic forms or totally harmless substances. Contamination sites can have mixtures of different contaminants; thus, it is useful to combine technologies to improve and enhance their remediation capacities. Remediation technologies can be applied directly in the contaminated site: through in situ remediation or, in special facilities to perform the cleaning up, ex situ. In both cases, the cleaning up or removal of the contaminants are achieved through physical, chemical, or biological methods.

Most methods for cleaning soils and ground- and surface water are either physical, chemical, or a combination of both. The first remediation solutions were excavation and confinement of toxic materials, but these are only partial solutions because they do not reduce the concentration of the contaminant but only isolate them. For soil, the simplest remediation technique is the removal of the contaminated soil ex situ by chemical means. Then the contaminated site is filled with noncontaminated soil. This method is applicable only in small areas, and sometimes the soil is only partially removed and mixed with clean soil, which dilutes the contaminant that can be naturally attenuated (Yao et al. 2012). For contaminated water, the simplest method is pump and treat, where the contaminated groundwater is extracted and treated before being reintegrated into the ground. This in situ method is practical and very much used, but it does not completely eliminate the contaminants. The process takes a long time and does not work on contaminants adsorbed into the soil particles. Thus, it is usually combined with other remediation techniques (Khan et al. 2004). Capping is also an in situ technique that isolates more than remediates the contaminated places. A clean layer of unreactive material such as gravel, sand, or rocks is deposited above the contaminated area to cover and isolate it. Different adsorbent materials are added to the cap composition to better prevent the spreading of the xenobiotics to the surrounding soil or water (Gomes et al. 2013).

Air sparging is a useful technology for the remediation of underground water contaminated with volatile organic compounds. It is the injection of air with pressure below the contaminated area; when the air passes through the soil, it can strip the contaminant away or increase the degradation caused by microorganisms that inhabit the soil due to increase in oxygen (Khan et al. 2004; Reddy 2008).

Soil flushing and soil washing use water and solvents to clean soils and groundwater through in situ remediation processes. The nature of the solvents used depends on the contaminant to be removed, semi-volatile organic compounds, petroleum and its derivatives, heavy metals, pesticides, PAHs, and polychlorinated biphenyls. The solution passes through the contaminated area, removing the noxious agents. The solution is recovered and further treated to dispose the contaminants and return the cleaned water (Yao et al. 2012; Lim et al. 2016; Khalid et al. 2017).

Contaminated water is also treated with permeable reactive barriers. The principle is to let the contaminated water pass through a wall constructed with the appropriate material to retain and/or degrade the contaminants. This technique is applicable to groundwater and surface water, and the success of it depends on the material used to construct the barrier. The most common reactive materials are zerovalent iron, carbon-based materials as activated carbon and plant-derived biomass, alkaline-complexing agents, metal oxides, zeolite (Thiruvenkatachari et al. 2008), and more recently carbon-based nanomaterials and graphene in 3D architecture to better adsorb a variety of organic and inorganic water contaminants (Chen et al. 2015a). Moreover, there are reactive permeable barriers that include microorganisms to degrade the contaminants. These are known as permeable reactive bio-barriers.

Among the chemical techniques to clean contaminated soils in situ and ex situ, electrokinetics is one of the most versatile. It has been used to clean soils contaminated with heavy metals, oil and petroleum derivatives, and other organic contaminants. The basic principle consists in the application of a low-intensity current generated by electrodes inserted in the contaminated area in electrolyte wells. The electric current promotes the mobilization of ions and metals by electrophoresis, electromigration, or electroosmosis. This technique has been optimized by the addition of suitable electrolytes to improve its efficiency, such as surfactants or acidic solutions, and in combination with permeable reactive bio-barriers (Gomes et al. 2012; Lim et al. 2016; Mena et al. 2016).

Various soil remediation techniques employ temperature increase to solidify/immobilize, volatilize, or decompose the contaminants.

Vitrification technology is useful to treat soils contaminated with inorganic pollutants such as heavy metals and radionucleids. Electrodes are used to apply electric energy, which promotes increase in temperature. The organic contaminants are volatilized, and the inorganic materials are molten at temperatures between 1000 and 2000 °C. Soil commonly contains silica, which functions as a vitrifying agent; during the cooling of the soil, the materials form a very resistant glass. The vapor produced is collected and treated (Khalid et al. 2017).

Soil contaminated with petroleum derivatives and other industrial solvents can be treated with soil vapor extraction to remove volatile organic compounds. Fresh air is injected into the soil, and a vacuum extraction system is installed to collect the gas, which is further treated. This technique is used in combination with other techniques, like increasing temperature to remove semi-volatile compounds or air sparging (Khan et al. 2004; Lim et al. 2016).

Thermal desorption is a technology used to clean soils contaminated with organic contaminants, oil, and petroleum-derived contaminants and utilizes high (320–560 °C) or low (90–320 °C) temperatures. The vapor produced is collected, separated into water and solvent components, and further used as recycled components or incinerated. The recovered water can be discharged or reused to cool the system (Yao et al. 2012; Gomes et al. 2013).

Despite great research on and the improvement of physical-chemical remediation technologies, there are disadvantages in their use. Some of those applied in situ require the addition of chemical agents to the soil (soil washing and flushing and electrokinetics), which can lead to noncontaminated areas or can alter the chemical composition of soil, rendering it unfit for agriculture or natural preservation. In situ and ex situ technologies require large amounts of energy to function, and some of them, like incineration or thermal adsorption, generate gases that need to be further treated to really eliminate the noxious agents. Thus, alternatives to these remediation techniques have been developed, and the use of living organisms to remove, transform, or degrade xenobiotics is now called bioremediation or biological remediation. The biological agents that are used to perform bioremediation are naturally occurring microorganisms that can degrade or even mineralize many xenobiotic compounds. Most of these treatments are conducted in situ and with techniques that have a much lower cost than physical or chemical remediation. The only disadvantage of bioremediation is that in many cases, the treatment can last for months or even years.

In the next section, we will review in more detail the bioremediation techniques developed so far.

5.3 Biological Treatments

As physical and chemical remediation of xenobiotics is costly and most of the time inefficient, bioremediation has attracted attention in recent years. Bioremediation is the treatment of xenobiotic wastes with living organisms or their parts (enzymes, cell walls, secreted polysaccharides, etc.) (Ortiz-Hernández et al. 2011; Hlihor et al. 2017). A huge diversity of organisms has been used to alleviate the effects of xenobiotic compounds in the environment, and thus it seems a promising alternative. Bioremediation is environmentally friendly, low cost, and many times more efficient than physical or chemical remediation since it usually converts the xenobiotic compounds into CO_2 and water. Bioremediation may be applied in situ or ex situ and can be achieved through two main techniques: biostimulation and bioaugmentation. In the first case, specific nutrients are provided into the contamination site so specific native microbiota can grow, cometabolizing the xenobiotic compounds. In the second case, native or foreign microorganisms or plants are introduced into the polluted habitat to degrade the pollutants (Alegbeleye et al. 2017). In this situation, especially in ex situ treatments, even transgenic microorganisms can be tested (Balcázar-López et al. 2016). The term bioremediation came as early as 1928, when Gray and Thronton discovered naturally occurring microorganisms that could degrade BTEX (benzene, toluene, ethylbenzene, and xylene) in soil. Here we will discuss three main groups of organisms that have been extensively studied and used for bioremediation.

5.3.1 Bacteria

One of earlier reports of a bacterium capable of degrading benzene derivatives (BTEX) was published by Williams and Murray in 1974 (Williams and Murray 1974). Later it was described that this strain of *Pseudomonas putida* carrying the TOL plasmid possessed an enzymatic route to degrade these compounds and use them as carbon source (Worsey and Williams 1975).

Since then, a big number of different bacterial species have been proved to degrade a wide variety of different xenobiotic compounds. Among the most studied are members of the genera *Mycobacterium*, *Pseudomonas*, *Alcanivorax*, *Burkholderia*, *Rhodococcus*, *Aeromonas*, *Arthrobacter*, *Micrococcus*, *Streptomyces*, *Bacillus*, *Sphingomonas*, *Cellulomonas*, *Micrococcus*, *Marinobacter*, *Haemophilus*, *Xanthomonas*, *Acinetobacter*, *Enterobacter*, *Corynebacterium*, etc. These genera can mineralize polycyclic aromatic hydrocarbons (PAHs), pesticides, and azo dyes and remove or change the redox state of heavy metals.

Although the degradation of many xenobiotics via oxidases has been better studied in fungi, ligninolytic-like enzymes have also been found in bacteria. Enzymes called yellow laccases have been described, but they show a lower redox potential toward their substrates than true fungal blue laccases (Valderrama et al. 2003; Riva 2006). Bacteria typically do not show peroxidase activity; however, another kind of peroxidase activity (by proteins nonhomologous to the fungal ones), known as dye-decolorizing peroxidases, has been described and studied (Van Bloois et al. 2010). In general, these enzymes are unspecific oxidases that can activate many xenobiotic compounds through the production of free radicals for their further mineralization or polymerization, rendering them nonbioavailable. Also, enzymes involved in oxidative stress such as catalases and superoxide dismutases have been involved in PAH degradation (de Gonzalo et al. 2016).

For pesticides such as organophosphate or carbamate compounds (ethyl or methyl parathion, coumaphos, carbofuran, carbaryl, etc.), several hydrolytic enzymes have been described (Singh et al. 2017). Although these compounds may also be oxidized, the hydrolytic route via phosphotriesterases is preferable since it will not produce toxic intermediate quinones (Ortiz-Hernández et al. 2011).

Heavy metals, on the other hand, cannot be degraded. The means for their bioremediation consists primarily in adsorption into the cell wall, compartmentalization in vacuoles or other organelles in eukaryotes, or changing their redox state to less soluble forms and thus making them less bioavailable. Usually, for these xenobiotic elements, plants are the best option, but a striking example of biostimulation is the capacity of heavy metals to transform into less toxic forms with the use of *Geobacter sulfurreducens* (Hernández-Eligio et al. 2017). In this system, acetate is pumped into the ground that is polluted by heavy metals (this has been especially efficient for manganese, uranium, or chromium) so that the anaerobe *G. sulfurreducens* can "respire" this substrate and transfer electrons to metals, converting them into less soluble forms that make them unavailable. The striking feature here is that, through this process, an electrical current is generated and conducted through specialized pili, and so this system is also being studied as a bioenergy-generating alternative.

5.3.2 Fungi

Fungi have been one of the most promising bioresources for bioremediation (especially white-rot fungi, pertaining to the *Basidiomycota*) since they produce extracellularly a plethora of oxidative and hydrolytic enzymes (Baldrian 2008). Fungi are eukaryotes that grow saprotrophically, forming hyphae, tube-like long cells that can form tight mats called mycelia. One of the advantages in using fungi for bioremediation in situ is the ability to cover and penetrate large surface areas by hyphae. Fungi have evolved to use complex carbon sources since they are sessile organisms and can decompose lignin, cellulose, and hemicellulose, which have different recalcitrant structures. Particularly, lignin, an amorphous phenolic polymer, shows some structural and chemical similarities to PAHs, organophosphate aromatic pesticides, and industrial dyes.

The most widely studied species belongs to the division *Basidiomycota*, although a large number of other species have been studied. Among the most studied genera, we can find *Trametes*, *Phanerochaete*, *Pleurotus*, *Bjerkandera*, *Coriollopsis* (*Basidiomycota*), and several different *Aspergilli*, *Trichoderma*, and *Fusarium* (*Ascomycota*). Although there are reports on yeasts (*Rhodotorula* spp., *Candida* spp., and *Yarrowia* spp.), these have been less studied.

Yeasts, ascomycetes and basidiomycetes have been also studied for the production of these aforementioned oxidases: laccases, peroxidases, lytic polysaccharide monooxygenases, and the intracellular monooxygenase cytochrome P450. Activities for all these are quite unspecific and thus can use as substrates large amounts of different compounds, such as PAHs, pesticides, endocrine disruptors, dyes, and even explosives such as trinitrotoluene. It is worth to note that the oxidases (especially laccase and peroxidases) produced by fungi have a much larger redox potential than their bacterial counterparts, thus being more efficient in xenobiotic degradation (Singh et al. 2017).

Other fungal enzymes involved in the mineralization of xenobiotic compounds are glucose oxidase, aryl alcohol oxidase, quinone oxidoreductase, and cellobiose dehydrogenase (Leonowicz et al. 1999).

Fungi can also be used for bioremediation of soil and water polluted by heavy metals (Abbas et al. 2014). Several mechanisms are used by these organisms that give an advantage over bacteria to control heavy-metal-polluted environments. The main mechanisms for heavy metal control is probably adsorption into the cell wall (which presents many carbonyl groups to which heavy metals can bind), and this is also true for bacteria and algae. These organisms can also conjugate heavy metals in several organic molecules such as glutathione or organic acids. However, fungi are eukaryotes that have cell compartments, and, in many cases, compartmentalization of the metals in different organelles (vacuole, endoplasmic reticulum, or the Golgi apparatus) is achieved to contend with the toxicity caused by the metals. Furthermore, fungi express specific proteins called metallothioneins, which are cysteine-rich proteins that can also "trap" these xenobiotics in the Golgi apparatus (Siddiquee et al. 2015).

In conclusion, bacteria are probably the most studied and used microorganisms for bioremediation of xenobiotic compounds, but fungi are promising bioremediator agents since they grow fast, can encompass large areas (e.g., be placed in large filters), and produce powerful and robust extracellular enzymes (Prasad 2017, 2018). Furthermore, in many cases, they can withstand harsher conditions than bacteria.

5.3.3 Phytoremediation

Plants are also very versatile organisms because they are sessile and thus have evolved to withstand a plethora of environmental challenges than other organisms can evade by running away from biological, chemical, or physical threats.

Bioremediation with plants has been mainly applied to alleviate pollution by heavy metals, but it has also proven useful with other kinds of xenobiotics, such as PAHs, pesticides, dyes, etc. (Rasmussen and Olsen 2004; Lin et al. 2005; Dixit et al. 2015; Tripathi et al. 2016). Plants use several mechanisms for the bioremediation of different compounds: phytovolatilization, phytostabilization, phytodegradation, and rhizodegradation (Arslan et al. 2017). The mechanism applies mainly to volatile compounds such as BTEX, which consists of the plant absorb such pollutants and transferring them to the air through stomata. However, this only takes the pollutant from one place to another, so no real remediation occurs.

Phytostabilization refers to the immobilization of the pollutants in the plant. Mostly, the toxic compounds are adsorbed into the roots, or by the root system, and its faith is then decided, depending on whether they can be degraded or just accumulated. In general, plants are able to absorb easily nonpolar contaminants such as PAHs or pesticides. The lipid content of the plant tissue is crucial for the plant to be able to absorb these kinds of xenobiotics (Goodin and Webber 1995).

Phytodegradation of organic pollutants has been poorly studied, although it is controversial. However, we must not forget that plants produce peroxidases and laccases that could act as a starting point for the degradation of PAHs, pesticides, and dyes (Günther et al. 1996).

On the other hand, rhizodegradation is the best studied and most successful mechanism. It involves the participation of plant-associated microorganisms that live in close vicinity with plant roots. Even if the microbes can many times degrade pollutants by themselves, the rhizospheric environment greatly enhances its efficiency. In some scenarios, microbes cannot mineralize xenobiotics without the plant counterpart (Grosser et al. 1995; Chen et al. 2015b; Hong et al. 2015).

In the rhizosphere, plants offer nutrients and residency to the microbial communities that live in the vicinity. As payback, some of these microbes often promote plant growth and develop tolerance to abiotic stress and resistance to phytopathogens for the plant. There are two main classes of rhizospheric interactions: surface root colonization and endophytic microorganisms penetrating the plant tissues and residing within the plant (Compant et al. 2010). The beneficial effect of microorganisms to the plant is given by the fact that microbes contribute to nutrient uptake by fixing nitrogen; producing siderophores (which can provide Fe); some excrete organic acids that dissolve insoluble minerals containing phosphate, calcium, and other essential nutrients; the production of plant hormones such as auxins and gibberellins; and enzymes that degrade ethylene, a hormone that stops plant growing in stressful situations. These interactions also induce both induced systemic resistance (ISR) and systemic acquired resistance (SAR), which protects the plant against pathogens (fungi, bacteria, and viruses) through the expression of proteins such as defense- and pathogen-related proteins (often chitinases and glucanases that degrade the cell wall of the pathogen). Finally, pH changes in the soil and other factors induce the response to oxidative stress, which protects the plant against drought, heat, cold, and other abiotic stresses (Brotman et al. 2013; Pelagio-Flores et al. 2017). Furthermore, as mentioned before, fungi produce a whole set of extracellular enzymes that are able to degrade or transform persistent organic pollutants, so the combination of plant and fungi for bioremediation looks very promising.

Among the most studied endophytic interactions is that of *Trichoderma* spp. with a whole different set of plants, from *Arabidopsis thaliana* to agroeconomical important plants such as cucumber, tomato, and beans (Salas-Marina et al. 2011; Dildey et al. 2016). Other fungi that also have been widely studied are those that form arbuscular mycorrhizal symbiosis. These microorganisms can also penetrate the plant tissues and help nurture the plant, especially in the acquisition of nitrogen and phosphate.

Many groups are now studying the potential of the plant-microbe associations to implement bioremediation strategies that have been found to be very promising. For example, up to 49% of hydrocarbon from petrol was removed in a consortium formed by oat (*Avena sativa*), *Acinetobacter* sp. (bacteria), and *Rhizophagus intraradices* (formerly *Glomus intraradices*) (an arbuscular mycorrhizal fungus) (Xun et al. 2015). Other cases of success were achieved by using common bean (*Phaseolus vulgaris*) mixed with N-fixing bacteria *Rhizobium* sp. to remove the pesticide atrazine from agricultural soils (Madariaga-Navarrete et al. 2017). Salami and coworkers (Salami et al. 2017) also obtained good results from using *Trichoderma harzianum* and the AMF fungus *Funneliformis mosseae* (formerly *Glomus mosseae*) for the treatment of *Capsicum annum* L. plants irrigated with water from a mining site.

In conclusion, bioremediation is a potentially effective strategy for the remediation of soils and waters polluted with xenobiotics. It is an emerging low-cost technology that still has to be explored but that has already proven to be efficient since very high percentages of persistent organic pollutants or heavy metals have been shown to be removed.

5.4 Phytoremediation and Fungi: Cases of Study and Perspectives

Mycorrhizoremediation has been identified as an enhanced form of phytoremediation. Fungi are very important organisms in the colonization of different plants. It has been suggested that glomalean fungi are essential in the colonization of briophyte-like land plants (Simon et al. 1993). Arbuscular mycorrhizal fungi are one of the oldest fungi in nature, and they establish symbiotic relationships with approximately 80–90% of land plants (Brundrett 2002). These fungi when associated with plants, improve the plants' nutrition, root development, productivity, and health. The fungus–plant interaction is the base of the mycophytore mediation.

Specifically, AMF fungi have been largely studied with respect to their ability to stabilize and sorb heavy metals in soils and also during their interaction with plants (Khan 2006). They produce glomalin, a related soil protein (GRSP) with properties to chelate metals in different environments. Some authors have proposed that glomalins immobilize heavy metals like a filter in the soil/hypha interface, and then they facilitate metal internalization into mycorrhizas. Thus, in association with roots, AMF fungi are crucial to phytostabilize heavy metals in soils. Even though there are many studies describing the biotechnological potential of AMF to develop phytoremediation systems (Khan 2006), few works critically analyze the molecular mechanisms involved in the phytostabilization of heavy metal by the AMF plant system. This represents a challenge for further study and at the same time should be considered a perspective in the field. The full understanding of these mechanisms will allow a better comprehension of the ecological role of glomalean fungi and, in consequence, the improvement of new phytoremediation systems. AMF also plays an important role in the aggregation of soil particles, because of which they produce glycol-soil-proteinaceous substances (glomalins) (Rillig et al. 2003; Sharma et al. 2017). These compounds have been poorly studied, and only little is known about their potential to bind heavy metals. Another unknown aspect is glomalin interaction with plants and the microbiota present in the soil. Further studies are required to establish the specific physiological and ecological roles of these proteins. But glycol-soil-proteinaceous substances are unique proteins secreted by AMF involved in the mycoremediation of heavy metals. Surely, other proteins with an important role in the sorption and sequestration of toxic elements would exist, not only metals but also organic xenobiotics.

Many studies to explore the potential applications of phytoremediation of heavy metals have been addressed with AMF. Greenhouse pot experiments were conducted using *Glomus versiforme* to determine its contribution to cadmium hyperaccumulation by *Solanum nigrum*. The *G. versiforme* inoculation has a significant effect on the extractable Cd concentrations and also enhanced acid phosphatase activity and phosphorous acquisition. This fungus supported the high growth of *S. nigrum* (Liu et al. 2015). Other *Glomus* species have exhibited good potentials for phytoremediation. *Rhizophagus intraradices* (previously known as *Glomus intrara-*

dices) enhanced zinc adsorption during its interaction with tobacco plants (Audet and Charest 2006), and *Funneliformis mosseae* (previously known as *Glomus mosseae*) allowed high removal of lead in association with vetiver roots (Wong et al. 2007; Punamiya et al. 2010). *Claroideoglomus etunicatum* (previously known as *Glomus etunicatum*) showed capabilities to reduce Ni concentration in the roots of *Sorghum vulgare* and enhanced plant growth, contributing to nickel phytostabilization (Amir et al. 2013). In general terms, mycorrhizal fungi represent a great friendly alternative for phytoremediation.

There are many works evaluating the biotechnological potential of mycorrhizal fungi for phytoremediation, but only few studies test these potentialities at a greenhouse scale. The major challenge is to transfer the laboratory scale results to the field, where other microbial communities might be playing a role in the process. Thus, mycophytoremediation deserves further efforts to generate novel knowledge to accelerate the application at real-time scale of those results generated at laboratory level and, therefore, attain improvement in mycorrhizal fungi biotechnology (Khan 2006).

Endophytic fungi also represent good candidates for phytoremediation. They play a crucial role in several processes such as organic and inorganic transformations, element cycling, rock and mineral biotransformations, bioweathering, mycogenic mineral formation, fungal–clay interactions, metal-fungal interactions, as well as organic compound–fungal interactions. These elements suggest that endophytic fungi could improve phytoremediation efficiencies (Deng and Cao 2017). This is another area with big opportunities to generate novel knowledge related to mycophytoremediation because has exhibited a discreet advance in recent years. As Deng and Cao pointed out, the role of endophytic fungi in phytoremediation has been poorly studied (Deng and Cao 2017). More attention should be concentrated on this topic to describe in detail the physiological properties of endophytic fungi and their potentialities in phytoremediation. In this context, yeasts have been scarcely studied with few reports related to their effects on phytoremediation of heavy metals. However, some works describing the use of endophytic filamentous fungi in the phytoremediation of organic pollutants have been published. For example, the endophyte *Ceratobasidium stevensii* enhanced the degradation of phenolic acids when it was inoculated to watermelon plants. At the same time, it promoted plant growth, helped grow more stems, and enhanced leaf length (Xiao et al. 2010). Another study evaluated the infection of endophytic fungi in the grass species *Festuca arundinacea* and *Festuca pratensis* during the phytoremediation of hydrocarbons in an aged-contaminated soil. It was demonstrated that the endophytic fungi significantly contributed to the total degradation of petroleum hydrocarbon, as well as root formation in the grasses (Soleimani et al. 2010).

Fungi could also act as endophytic microorganisms since they can colonize roots. Endophytic microbes, especially fungi, establish intimate and symbiotic relationships with plant, and these interactions could contribute to the efficiency of phytoremediation. Thus, the mycoremediation implementation at field level needs a full understanding of the endophytic fungi–microbiota–plant interaction (Prasad 2017, 2018).

As petroleum-derivate-polluted soils are very common in many countries, fungi have also been used for phytoremediation of diesel at laboratory levels. It has been proved that diesel is a potent inhibitor of growth for a wide range of plants. Some plants exhibit better growth in the presence of diesel when they are inoculated with AMF (Alarcón et al. 2006; Joner and Leyval 2003; Hernández-Ortega et al. 2012). During the interaction between *Melilotus albus* and *Glomus* Zac-19 in a soil contaminated with diesel, *M. albus* showed better growth, and diesel was significantly degraded when *Glomus* Zac-19 was used in the experiment (Hernández-Ortega et al. 2012). It was also demonstrated that *Glomus* Zac-19 significantly reduced diesel toxicity on the plants because of the fungus-enhanced plant biomass, nutrient sequestration, and the total antioxidant activity involved (Hernández-Ortega et al. 2012).

Another AMF has been investigated for the phytoremediation of cadmium and organic pollutants, such as decabromodiphenyl ether (BDE-209) (Li et al. 2018). *Funneliformis mosseae*– and *Rhizophagus intraradices–Solanum nigrum* interactions revealed an improvement of shoot biomass and the cadmium contents in shoots in comparison with uninoculated plants (Li et al. 2018). Thus, fungi are ideal for phytoremediation since they can coremove both organic and inorganic pollutants. Additional efforts are necessary in the investigation of fungi-based phytoremediation of soil cocontaminated with heavy metals and organic compounds since studies on this have been scarce and poli-polluted environments are more frequently found. These studies will find new insights in order to establish new/novel cost-effective, efficient, and environmentally friendly mycophytoremediation strategies for the removal of multiclass pollutants.

Interested in the development of mycophytoremediation technologies, Mohsenzadeh et al. (2010) studied some fungal–plant interactions in petroleum-polluted soils. Seven plants showed tolerance and growth in the presence of petroleum (Mohsenzadeh et al. 2010). They were *Alhaji cameleron*, *Amranthus retroflexus*, *Convolvulus arvensis*, *Chrozophora hierosolymitana*, *Noea mucronata*, *Poa* sp., and *Polygonum aviculare*. Eleven fungi isolated from these plants, some endophytic fungi between them, tolerated 1% (v/v) of petroleum, while *Fusarium* species resisted 10% (v/v). The study demonstrated that plants of *P. aviculare* inoculated with *F. acuminatum*, *F. equiseti*, *F. reticulatum*, and *F. oxysporum* significantly alleviate the petroleum pollution in the soil in comparison to plant and fungi separately (Mohsenzadeh et al. 2010). While huge attention has been given to the investigation of fungi as a promising degrader agent, little attention is noted in analyzing the role and biotechnological potentialities in phytoremediation of fungal-plant systems in the bioremediation of hydrocarbons. On the other hand, no mycorrhizal fungi have received poor attention. Further researches and efforts should be considered to facilitate new knowledge related to mycophytoremediation using free-living fungi.

In conclusion, AMF exhibits an excellent potential in the phytoremediation of metals and organic pollutants. Figure 5.1 (Rajtor and Piotrowska-Seget 2016) shows the benefits derived from these types of fungi since they enhance nutrient and water acquisition, promote plant growth, facilitate the stabilization and aggre-

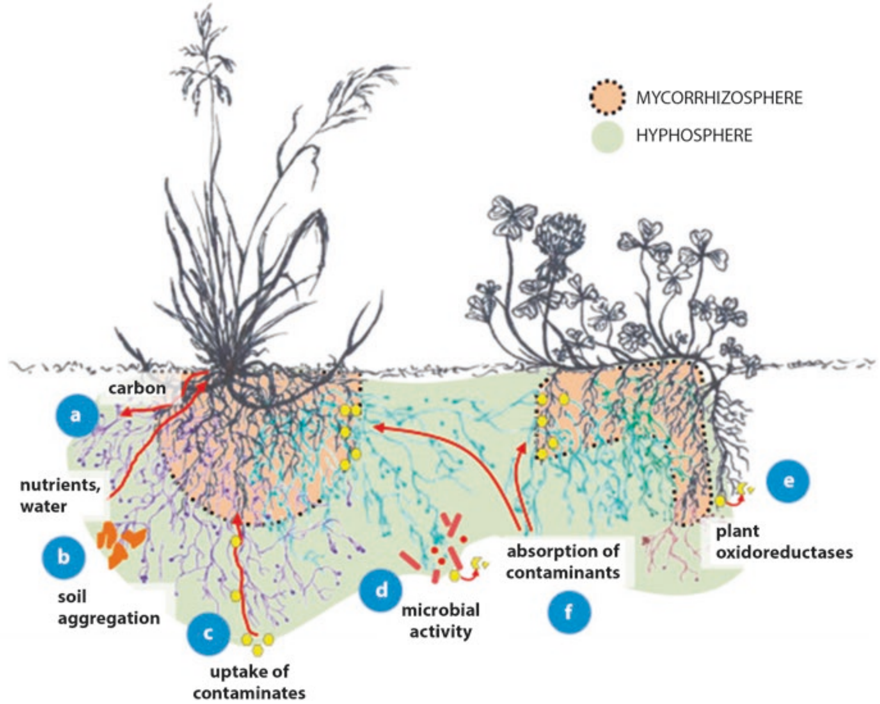

Fig. 5.1 AMF and its potential in phytoremediation. Fungal growth (**a**) facilitates the acquisition of nutrients and water, (**b**) improves the stabilization and aggregation of soil particles, (**c**) promotes plant growth, (**d**) participates in carbon degradation using oxidoreductases and a huge battery of degrading enzymes, (**e**) changes the profile of root exudations and the microbiota composition, (**e**) increases the adsorption area in roots since it enhances the lipid content in the root system

gation of soil particles, allow the immobilization of organic pollutants, contribute to carbon degradation, stimulate root exudates and the proliferation of the rhizomicrobiota increasing enzymatic activities with a crucial role in the degradation of organic contaminants, and protect plants from oxidative stress (Rajtor and Piotrowska-Seget 2016).

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