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Mycoremediation in Soil

Francesca Bosco and Chiara Mollea

Abstract

The chapter reviews the most important researches on the use of micro- and macrofungi in the bioremediation of contaminated soils. In particular, the main classes of soil pollutants in Europe (heavy metals, mineral oils, polycyclic aromatic hydrocarbons (PAHs), monoaromatic hydrocarbons, phenols and chlorinated hydrocarbons (CHCs)), together with the emerging contaminants (i.e. endocrine-disrupting chemicals (EDCs) and pharmaceutical-personal care products (PPCPs)) are considered. A description of the fungal species (saprotrophic and biotrophic basidiomycetes) and biodegradative extracellular (laccases and class II peroxidases) and intracellular (cytochrome P450 monooxygenases and glutathione transferases) enzyme classes is reported. Moreover, the chemical-physical parameters that influence the biodegradation process are examined, and the biostimulation and bioaugmentation strategies are described. A specific attention is paid to the microcosm studies, at the laboratory scale, which are an essential approach to evaluate the feasibility of a biodegradation process.

Keywords: mycoremediation, filamentous fungi, mushroom, microcosm, biostimulation, bioaugmentation, laccases, peroxidases, cytochrome P450 monooxygenases, glutathione transferases

1. Introduction

The contamination of soil, water and air by toxic chemicals represents one of the major worldwide environmental problems. From this point of view, the European Union (EU) is paying attention to the improvement of soil protection and recovery and to the prevention of soil contamination, since there are still many historical and new contaminated sites that require remediation [1, 2]. The main classes of soil pollutants in Europe have been reported in [3].

Bioremediation is a simple and cost-effective method that, in the last decades, has received worldwide a particular attention. The general term “bioremediation” indicates the use of living organisms (i.e. bacteria, fungi, algae and plants) in the detoxification of polluted soils and wastewaters. In a bioremediation process, organic and inorganic hazardous substances may degrade, accumulate or immobilize, resulting in a significant reduction of the contamination level.

In the last decay, the role of fungi in bioremediation has been increasingly recognized [4, 5]. About this, various authors have highlighted the ability of fungi, mainly saprotrophic and biotrophic basidiomycetes, to degrade or to transform toxic compounds [6, 7]. Mycoremediation is the bioremediation technique which employ fungi in the removal of toxic compounds; it could be carried out in the presence of both filamentous fungi (moulds) [8] and macrofungi (mushrooms) [9, 10]. Both classes possess enzymes for the degradation of a large variety of pollutants [11, 12].

Fungi are well known for their ability to colonize a wide range of heterogeneous environments and for their ability to adapt to the complex soil matrices, also at extreme environmental conditions. Furthermore, they can decompose the organic matter and easily colonize both biotic and abiotic surfaces [13, 14].

Filamentous fungi show some peculiar characteristics that make them more advisable in soil bioremediation than yeasts and bacteria [14, 15]. The most important are the type of growth (i.e. the development of a multicellular mycelial network) suited to soil colonization and translocation of nutrients and water, the production of many bioactive compounds and extracellular enzymes and the unique capability to co-metabolize many environmental chemicals [16].

Mycoremediation represents thus a biological tool to degrade, transform or immobilize environmental contaminants.

The state of the art of soil mycoremediation is reviewed in the present chapter. A particular attention is given to the fungal species and enzymes involved in the biodegradation processes, together with the classes of toxic compounds that could be biodegraded. Bioremediation strategies (i.e. biostimulation and bioaugmentation) and significant examples of microcosm and field studies are also discussed. Finally, the application of mushrooms as emerging technology in soil mycoremediation is reported.

2. Important fungal species involved in biodegradation

The most suitable fungi to be used in soil remediation are basidiomycetes and, in particular, the ecological groups of saprotrophic and biotrophic fungi [17].

The saprotrophic basidiomycetes, which use dead organic matter as a carbon source, include the wood-degrading fungi. Among them, white-rot fungi (WRF) are considered for the leading role in biodegradation [18]. WRF can degrade efficiently both lignin and cellulose biopolymers till the complete mineralization [19], thanks to the production of an extracellular enzymatic complex, which comprehend lignin peroxidases (LiPs), manganese-dependent peroxidases (MnPs), versatile peroxidases (VPs), laccases, H₂O₂-generating oxidases and dehydrogenases, produced during the idiophase, usually under nitrogen depletion.

Some of the most representative WRF, able to degrade pollutants, include *Phanerochaete chrysosporium*, *Pleurotus ostreatus*, *Trametes versicolor*, *Bjerkandera adusta*, *Lentinula edodes*, *Irpex lacteus*, *Agaricus bisporus*, *Pleurotus tuber-regium* and *Pleurotus pulmonarius* [20, 21]. Among these fungi, *Phanerochaete chrysosporium* has been the most investigated for its ability to degrade toxic or insoluble compounds to CO₂ and H₂O, more efficiently than other fungi. In 1985, for the first time, Bumpus et al. proposed the application of *Phanerochaete chrysosporium* in bioremediation studies, and the fungus became a model system in the mycoremediation field [22].

The biotrophic basidiomycetes comprehend ectomycorrhizas which obtain the carbon source from a mutualistic plant partner: the fungal hyphal network envelopes the root and penetrates between the cells of the root cortex [17]. Ectomycorrhizal fungi (ECM) can assemble and recycle the nutrients from the organic matter of the soil [23]. ECM comprehends about 10,000 fungal species; the most representatives are *Amanita* spp., *Boletus* spp., *Gautieria* spp., *Hebeloma* spp., *Lactarius* spp., *Morchella* spp., *Suillus* spp. and *Rhizopogon* spp. [16, 24]. ECM fungi secrete enzymes to get nutrients by means of the degradation of molecules in the soil organic matter. ECM possesses extracellular and cytosolic enzymes which attack molecules containing N and P atoms. Hydrolytic enzymes comprehend β -glucosidases and phosphatases, while the oxidative ones are peroxidases and phenol oxidases [25]. The ECM fungi application is important in habitat where the litter

layer is restricted and consequently ligninolytic enzymes, typical of wood fungi, are not so efficacious; in this contest, ECM fungi are able to produce enzymes to sequester nutrients directly from the soil. The same enzymes allow ECM to degrade many persistent organic pollutants [26].

Most of the biodegradation studies at the laboratory and field scale are concerned to microfungi, but in the last years, much attention has been given to mushrooms which are broadly present in soil and also easily soil-cultivated [27]. Bioremediation by macrofungi basidiomycetes is reported by [28] to be advantageous because, together with remediation, soil is enriched with organic matter and nutrients and plant growth results enhanced. These macrofungi are potent degraders thanks to the secretion of the same non-specific enzymes (LiP, MnP and laccase) described for the wood-degrading fungi and, for this reason, are interesting in the bioremediation field. At the same time, they grow to a great extent producing high biomass quantities, in particular when cultivated on carbon sources, such as straw or sawdust [29]. The mushroom biomass can be a protein source or can contain biologically active compounds such as phenols with antioxidant activity [12, 30]. Furthermore, mushroom biomass can be applied in biosorption treatment thanks to its ability to accumulate ions and xenobiotics from contaminated soils [31].

3. Toxic compounds degraded by fungi

The biodegradation capability of different hydrocarbon classes such as mineral oils, polycyclic aromatic hydrocarbons (PAHs), monoaromatic hydrocarbons and chlorinated hydrocarbons (CHCs), together with phenols, was demonstrated for many fungal species [17]. Moreover, the possibility to decrease the risk associated with heavy metals, metalloids and radionuclides in soil has been described [16].

Cd, Cr, Hg, Pb, Cu, Zn and As are the most common heavy metals found in soil. In the EU, more than 80,000 contaminated sites are counted. Heavy metals can be generated by natural processes, like the metal-enriched rock erosion, and anthropogenic activities (e.g. mining, smelting, fossil fuel combustion, waste disposal, corrosion and agricultural practices) [32, 33]. Heavy metals that enter the environment can be transported or transformed by means of photo-, chemical- or biodegradation; moreover, they can also be biotransformed [34]. Fungi are potential heavy metal accumulators; in particular basidiomycetes mushrooms can uptake heavy metals from soil by means of their mycelia and accumulate them in the fruiting bodies, irrespective of their age [35]. As reported by [10], species of *Agaricus*, *Amanita*, *Cortinarius*, *Boletus*, *Leccinum*, *Suillus* and *Phellinus* are some of the mushroom applicable for the mobilization/complexation of different heavy metals in soil.

In the EU, mineral oils, together with heavy metals, represent the main source of soil contamination, significantly greater than 60% of the total contaminants. Mineral oils, refined from crude petroleum oil, are a group of various hydrocarbons, straight and branched-chain paraffinic, naphthenic and aromatic ones, with 15 or more C numbers [2]. They can be used for the preparation of lubricant products (e.g. engine oils or hydraulic fluids) or “non-lubricant” ones (e.g. agricultural spray oils). Their industrial application is at a large scale, and the soil contamination can occur during transport, storage or refining or also for accidental leakages [36]. Hydrolases, dehydrogenases and membrane-bound cytochrome P450 enzymes constitute the fungal hydrocarbon-degrading system [37]. Fungal species belonging to *Rhizopus*, *Paecilomyces*, *Alternaria*, *Mucor*, *Gliocladium*, *Aspergillus*, *Fusarium*, *Cladosporium*, *Geotrichum*, *Penicillium* and *Pleurotus* are capable of utilizing crude oil as the sole carbon and energy source [37–39].

Polycyclic aromatic hydrocarbons (PAHs), molecules with multiple carbon rings, derive from the incomplete combustion of organic materials. Their origin can be both natural (e.g. open burning, natural losses of petroleum and volcanic activities) and predominantly anthropogenic (e.g. residential heating, coal gasification, carbon black, activities in petroleum refineries). PAH contamination corresponds to 13%: these compounds tend to bound to soil particles and to remain absorbed [40]. Both ligninolytic and non-ligninolytic fungi are able to degrade PAHs by means of the extracellular lignin-degrading enzymatic system, which contribute to the first attack on PAHs, and of the P450 monooxygenase [41]. Apart from the model *P. chrysosporium*, species belonging to *Aspergillus*, *Penicillium*, *Rhizopus*, *Fusarium*, *Cladosporium* and *Trichoderma* are capable of degrading PAHs [42].

Another group of crude petrol-derived hydrocarbons, which represent the 6% of soil contaminants, is that of monoaromatic hydrocarbons, and in particular those grouped in the acronym BTEX (benzene, toluene, ethylbenzene and xylene). Fungi are efficient in aromatic hydrocarbon degradation, as for PAH degradation, thanks to the ligninolytic enzymatic system. WRF, such as *P. chrysosporium* and *Trametes versicolor*, are reported to be good BTEX degraders together with soil and mycorrhizal fungi [43].

Phenols consist of one or more aromatic rings with hydroxyl functional groups; they are present in the waste streams of almost all the phenolic-using industries (e.g. chemical, paper, food and textile industries) and contaminate the soil as leachates or particulate matter [44, 45]. The percentage of soil contamination is one of the lowest, being around 4% [33]. The biodegradation of phenols is mainly concerned to the production of phenol oxidase enzymes (laccases, tyrosinases and peroxidases) by basidiomycetes: they act on phenols and incorporate one or two atoms of oxygen [46, 47]. Due to the production of these multiple oxidative enzymes, *Trametes* spp., *Lentinus* spp., *Pleurotus* spp. and *Ganoderma* spp. are some of the most efficacious fungal species involved in phenol compound biodegradation [48].

The soil contamination of CHCs is about 2%. These compounds contain Cl atoms substituted for hydrogen atoms normally bonded to a carbon. This group of chemicals comprehends highly toxic pollutants such as polychlorinated biphenyls (PCBs) and chlorinated pesticides, e.g., DDT [49]. As for PAH biodegradation, WRF have been intensively proposed as biodegraders of CHCs due to their unspecific oxidative enzymes. However, also non-WRF, in particular soil ascomycetes and zygomycetes, are able to enzymatically transform these pollutants; in particular, they have the advantage over WRF to tolerate neutral pH and adverse growth conditions [50].

In the last years, emerging contaminants have become of great interest [51]. Among them, the anthropogenic chemicals, endocrine-disrupting chemicals (EDCs) and pharmaceutical-personal care products (PPCPs) are relevant due to their biological effects on nontarget organisms; in particular, EDCs simulate or antagonize the endogenous hormone effects and are toxic to organisms also at very low concentrations. Estrone, 17 β -estradiol, 17 α -ethinylestradiol, bisphenol A and triclosan are the most detected and studied in soil. EDCs and PPCPs mainly enter the soil environment via irrigation with contaminated wastewater [52–54]. As reviewed by [55], ligninolytic fungi are able to transform EDCs allowing a reduction of the endocrine-disrupting activity or their ecotoxicity; moreover, these fungi are also reported to be able to degrade the heterogeneous class of PPCPs thanks to their broadly unspecific enzymatic systems [56].

4. Enzymes involved in biodegradation of toxic compounds

Since 1985, after the discovery of Bumpus [22] about the degradation potentialities of *P. chrysosporium*, a plethora of authors have described the fungal enzymatic

machinery and its role in the transformation of a wide range of organic pollutants in soils. Most of the enzymes are extracellular and allow to attack and then degrade large molecules into smaller units which can enter the cells for further transformations [57].

Extracellular laccases start ring cleavage in the biodegradation of aromatic compounds [8]. They are multicopper oxidases with low substrate specificity and can act on *o*- and *p*-phenols, aminophenols and phenylenediamines thanks to a four-electron transfer from the organic substrate to molecular oxygen. The laccase-mediator systems (LMSs) have an effect on the electron transfer chain increasing the laccase substrate range [58].

Fungal peroxidases generate oxidants which initiate the substrate oxidation in the extracellular environment [8]. They belong to the class II peroxidases [59] and catalyse the oxidative conversion of various compounds utilizing H₂O₂ as electron acceptor. As previously reported, LiPs, MnPs and VPs are the main fungal high-redox class II peroxidases. They are involved in the biodegradation of the complex lignocellulose structure and, consequently, can degrade various organic substrates and transform some inorganic ones [46]. Fungi can also secrete the dye-decolorizing peroxidases (DyPs), which have oxidative and hydrolytic activities on phenolic and non-phenolic organic compounds [60]. Heme-thiolate peroxidases (HTPs) transfer peroxide-oxygen, from H₂O₂ or R-COOH to substrate molecules; in this group chloroperoxidases (CPOs) and the unspecific or aromatic peroxygenases (UPOs or APOs) are included. In particular, UPOs can mainly operate on heterogeneous substrates thanks to aromatic peroxygenation, double-bond epoxidation or hydroxylation of aliphatic compounds [59].

Intracellular detoxification pathways comprehend multigenic families of cytochrome P450 monooxygenases and glutathione transferases, mainly owned by wood and plant litter fungi but also by some symbiotic species [46]. These intracellular enzymes have functional roles in fungal primary and secondary metabolism.

P450 cytochrome monooxidases, heme-thiolate-containing oxidoreductases, can act on various substrates in stereo- and regioselective manner, needing O₂ for the reaction. They are activated by a reduced heme iron and add one atom of molecular oxygen to a substrate. Hydroxylation, epoxidation, sulfoxidation and dealkylation can occur and require NAD(P)H as electron donor [61].

Glutathione transferases are located in different cellular compartments and catalyse the nucleophilic attack of an electrophilic C, N or S atom in non-polar compounds by means of reduced glutathione (GSH). When electrophilic substrates are conjugated with GSH, they become more water-soluble. These enzymes have a wide substrate specificity and take part in the detoxification of different endogenous toxic metabolites and exogenous toxic chemicals [62].

5. Main parameters that influence mycoremediation

In general, chemical-physical characteristics of soil, such as pH, temperature, water content and redox potential, show a significant impact on the microbial growth and consequently on the success of a bioremediation process.

In particular, the biodegradation activity of the microorganisms depends on macro- and micronutrient availability in soil and on the presence of any other factor that influence the microbial metabolism, such as the contaminant type and concentration, and their bioavailability, toxicity and mobility [33].

A proper amount of nutrients for microbial growth is usually present in soil; nevertheless, nutrients can also be added in a functional form which serves as an electron donor to stimulate bioremediation process [63]. The biodegradation of a toxic compound mainly depends on the genetic characteristics of the

microorganism, in particular on both the extracellular and intracellular enzymatic systems [64]. The contaminant concentration directly influences the microbial activity: a high concentration may produce a variety of toxic effects on the different microbial classes, whereas a low concentration could not be enough to activate degradative enzyme synthesis. Filamentous fungi, able to form extended mycelial network and to synthesize a lot of aspecific enzymes, generally show a higher resistance to high contaminant concentration than bacteria [16]. Moreover, thanks to the low substrate specificity, the synthesis of degradative enzymes occurs also at low contaminant concentrations. The intracellular metabolic pathways involved in mycoremediation show remarkable similarities with those that regulate the secondary metabolism in fungi, in particular those of mycotoxin production [64]. Filamentous fungi which produce mycotoxins (e.g. *Aspergillus* and *Penicillium* spp.) exhibit the ability to degrade a wide variety of pharmaceutical compounds [65], among them the emerging pollutants EDCs [16, 66], ineffectively degraded by bacteria. The contaminant bioavailability is one of the most important factors that can be improved to optimize and accelerate the biodegradation; this fact has been demonstrated in the mycoremediation of aged PAH-contaminated soils [67]. The fungal ability to chemically modify or affect the contaminant bioavailability by means of biosurfactant production has been reported in different reviews [68, 69]. *Penicillium* and *Aspergillus* species have been reported to be biosurfactant producers [70, 71]. A wide range of microbial biosurfactant applications have been reported in the environmental protection field (e.g. enhancing oil recovery, controlling oil spills, biodegradation and detoxification of oil-contaminated soils) [69].

6. Biostimulation and bioaugmentation

Biostimulation and bioaugmentation are the two most developed approaches among the bioremediation techniques. Their main purposes are the reduction of bioremediation time and the achievement of a complete removal of contaminant [4].

In biostimulation, nutrients and electron exchangers are injected into the contaminated site in order to stimulate the degrading ability of indigenous microorganisms [72]. As regards lab-scale tests, nutrients are generally added as inorganic salts and as defined chemical species, while at the field scale, the nutrients are frequently added in the form of agro-wastes, organic wastes or inorganic fertilizers [63]. The main inorganic nutrients, usually added, are nitrogen and phosphorous, because the presence of organic toxic chemicals frequently induces an imbalance in the C:N:P ratio [73]. The main advantages of biostimulation approach are the low cost and the exploitation of indigenous microorganisms without the necessity of adaptation required by allochthonous species.

In bioaugmentation, allochthonous or enriched autochthonous microorganisms, able to metabolize a specific contaminant, are introduced in soil. In both cases, the homogeneous dispersion of the added biomass and its proliferation, in competition with native microorganisms, are the great challenges [63]. Moreover, bioaugmentation and biostimulation could be also coupled in order to further stimulate introduced biomass [74].

In fungal augmentation, high-quality inocula with high potentiality are necessary; consequently, specific methods have been developed for the production of fungal inocula. These inocula can be in the form of pelleted solid substrates, colonized by fungal mycelium, prepared from cheap agricultural and industrial by-products [4, 75]. Pelleted fungal inocula can be optimized in substrate composition to enhance fungal growth, degradation abilities and competitiveness against autochthonous soil microorganisms.

The bioaugmentation with autochthonous filamentous fungi for the cleanup of a historically contaminated site has been shown to be a successful bioremediation approach as described by [76]. These fungi were able to grow under nonsterile conditions and to degrade various aromatic hydrocarbons in the same contaminated soil.

In a recent review [77], the role of saprotrophic fungi in the biodegradation of xenobiotics and toxic metals in co-contaminated sites has been discussed along with the metabolic interactions between fungi and bacteria in a microbial consortium. Considering the occurrence of a mixed organic-inorganic contamination in brown field sites, the bioremediation mechanisms for combined pollution of PAHs and toxic metals by fungi and bacteria are also well documented [78].

7. Microcosm study at the lab scale

Microcosm studies are needed, before the in-field treatment, to evaluate microbial potential to degrade soil pollutants, the activity of the indigenous biomass and the most effective bioremediation strategy (i.e. biostimulation and/or bioaugmentation). In order to obtain information on the contaminant biodegradation in soil, the use of microcosms is a better approach than other kinds of laboratory tests [79]. Even if trials carried out at the lab scale do not always guarantee reproducible results on-site, due to chemical, physical and biological factors, they allow to verify the biodegradability of a certain compound. Hereafter, some of the most significant soil microcosm studies with fungi are reported.

One of the first studies, about PAH degradation in soil microcosm, was carried out with *P. chrysosporium* and *T. harzianum*, grown on wheat straw and then inoculated in naphthalene-contaminated soil. The biodegradation behaviour was monitored by means of naphthalene concentration measurement, CO₂ evolution as well as phytotoxicity tests [80].

Phanerochaete velutina and many litter-decomposing fungi (LDF) are potential degraders of soil organic matter. In the work of [81], they showed good growth, respiratory activity and MnP production on pine bark as co-substrate in microcosm. In the work of [82], the addition of *P. velutina*, cultivated on pine bark, to a PAH-contaminated soil was evaluated in microcosm and at the field scale. In the microcosm treatment (about 1 kg of soil), the bioaugmentation with fungi showed a positive effect on the biodegradation of the high molecular weight PAHs. On the contrary, in the field-scale experiment (about 2 tons of soil), carried out at lower starting concentration of PAHs, the degradation percentage (%) was similar in both the inoculated and non-inoculated soils.

The bioremediation of an aged PAH-contaminated soil in microcosm was demonstrated for an isolate of *Trichoderma reesei* [83]. The fungus metabolized benzo[a]pyrene in the presence of glucose as a co-metabolic substrate.

An isolate of *Chaetomium aureum* was able to halve the free Pb concentration in soil in about 2 months, irrespective of its association with indigenous microorganisms when inoculated in Pb-contaminated soil microcosms [84].

A microcosm study was conducted to optimize the degradation of weathered total petroleum hydrocarbons (TPH) in arid soils contaminated for more than a decade. Among fungi, *Aspergillus*, *Acremonium*, *Cryptococcus*, *Geotrichum* and *Penicillium* were the most widespread in these soils [85].

Different fungal strains (*Aspergillus*, *Fusarium*, *Rhizomucor* and *Emericella* spp.), isolated from a higher As contaminated agricultural soil, showed different detoxification mechanisms (biosorption/bioaccumulation and biovolatilization). They were able to reduce As contamination under in situ conditions as reported by [86].

In a study on bioremediation of petroleum hydrocarbons, a periodic biostimulation and bioaugmentation (PBB), by a single strain or a fungal consortium, was reported

as the best biodegradation strategy [87]. PBB maintained the enzymatic activities of a fungal co-culture (*Pestalotiopsis* sp., *Polyporus* sp. and *Trametes hirsuta*) and enhanced the biodegradation rate, in particular during the early stage of remediation [73].

The biodegradation activity of *Byssochlamys nivea* and *Scopulariopsis brumptii* was evaluated in agricultural soil microcosms contaminated with pentachlorophenol (PCP), added with solid urban waste compost [88]. A synergistic effect of compost and fungal strains was observed with a reduction of more than 95% of PCP after 28 days of incubation. The detoxification role of the two fungal strains in the contaminated soil was also confirmed by toxicity assays [89].

8. Mushrooms as an emerging issue in mycoremediation

Mushroom application in the bioremediation field could be considered as an emerging technology; nevertheless, a lot of scientific works have appeared in the last years.

The biodegradation potential of mushroom species in soil has been reviewed by [9]. In this chapter, the mycelial capability of hyperaccumulate chemical elements, in particular heavy metals and radionuclides, along with the nutritional potential hazards due to mushroom consumption has been extensively discussed.

The biodegradation of recalcitrant pollutants like PAHs by WRF, the bioremediation of soil contaminated with engine oil by *Lentinus squarrosulus* and the decontamination of soils polluted with cement and battery wastes using *Pleurotus pulmonarius* were also reported by [29].

Many works on the edible mushroom *P. ostreatus* have been published. The biodegradation of the carcinogenic secondary metabolite aflatoxin B₁ (AFB₁), produced by *Aspergillus flavus* on rice straw [90] and on maize [91], was reported for this species. The mycoremediation of heavy metal-contaminated soils by means of different *Pleurotus* species was also reviewed in the work of [92]. In general, *Pleurotus* spp. are reported to be able to accumulate high levels of heavy metals; each species is characterized by different sensitivities towards the different metals and their concentration.

In the review of [10], mushroom bioaccumulation of different potentially toxic trace elements (PTEs) in the fruiting bodies was reported for *Phellinus badius*, *Amanita spissa*, *Lactarius piperatus*, *Suillus grevillei*, *Agaricus bisporus*, *Tricholoma terreum* and *Fomes fomentarius*. The accumulation capability was higher than that of plants, vegetables and fruits.

The bioremediation of crude oil-contaminated soil by an unidentified *Agaricomycetes* was demonstrated in the work of [93]. The addition of 10% of spent mushroom compost (SMC) allowed to degrade petroleum hydrocarbons over a short period of time.

9. Conclusion

The capability of micro- and macrofungi to degrade organic pollutants and to decrease heavy metal concentration in soil is a matter of fact. The growth morphology in soil (i.e. extended hyphal network), the low specificity of extracellular enzymatic complexes and the possibility to use toxic compounds as the growth substrate make filamentous fungi more advantageous in bioremediation processes when compared to other microorganisms. However, in the design of a soil mycoremediation process, some important aspects have to be considered such as the choice of the appropriate fungal strain and the evaluation of its possible interaction with the contaminated soil microbiota. To this end, microcosm studies represent a useful and simple method which allows to evaluate the feasibility of a biodegradation process.

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