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Chapter X

Fundamental Fungal Strategies in Restoration of Natural Environment

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Abstract

Fungi play vital role in nutrient cycling by achieving process of decomposition of organic matter and breakdown of complex compounds in nature. This role of fungi coupled with their fundamental strategy of adaptation to various environmental factors may be used for designing systems to enable the elimination of biopolymers and man-made xenobiotics from the environment via biosorption. Soil contaminants like heavy metals, radionuclides, polyaromatic hydrocarbons-PAHs and chemicals used in agriculture that are toxic and carcinogenic agents could be diminished or removed by activity of fungal exoenzymes. In this review, special attention was paid to lignolityc enzyme system expressed by white-rot fungi recognized as successful agent in bioremediation of a large variety of chemicals that are, like lignin, relatively long lived in the environment. Furthermore, fungi are very important in natural cycling of metal ions due to their great accumulation potential for heavy metals (Pb, Hg, Cd) and radionuclides (^{137}Cs), implicating them as good bioindicators of the pollution in urban and industrial areas and in contaminated forest ecosystems. These processes in macrofungi are influenced by environmental factors like metal concentrations of soil and substrate, pH, organic matter and contamination by atmospheric deposition as well as fungal factors like fungal structure, biochemical composition, decomposition activity, development of mycelium and sporocarps or portion of fruiting body. Concentration of radionuclides in fungi is determined by the amount of radioactivity precipitation, concentration of stable - non radioactive or analogous element in soil, soil characteristics (mineral composition, pH) and its taxonomic and nutritional identity.

Macrofungi maintain ecological balances used as bioindicators or as remediation agents of contaminated environment. Also, edibility and medicinal properties are of a

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great importance for humans while majority of edible and medicinal mushrooms can accumulate high amounts of heavy metals and other environmental pollutants. This chapter summarizes relevant biological features of fungi (position in tree of life, nutritional strategy, enzyme systems), especially lignicolous macrofungi (mostly white-rot), as a basic tool for resolving physiological, ecological and biotechnological potential of fungi in changing polluted environment thus restoring the natural environment.

1. Introduction

Fungi are ubiquitous in natural environments representing one of the most important organisms in the world. This is not only due to their huge diversity and abundance, which are connected to their vital roles in ecosystem function, but also because of their influence on humans or human related activities. We recommend that potential application of fungi by humans should be preceded with the following actions: 1) taxonomical examinations, 2) impacts of pollutants contained in fruiting bodies (mycelium) of mushrooms on the environment, 3) heavy metal and radio-ecological analysis of terrain, microhabitats and fruiting bodies (mycelia) of fungal species.

1.1. Where do Fungi Belong?

It is supposed that the number of fungal species has been approaching almost 1,500.000, today, although only 5% of fungi is described [1]. Fungi represent a large, mysterious group of organisms for a long time while scientists are still working hard to find a proper place for them among the other groups of living organisms. Their specific characteristics and huge diversity confused scientists worldwide, often leading them in a wrong direction in classification. In the Linnaean (Carl von Linné) two Kingdom system of classification, fungi were included in the Plant Kingdom [2] according to their immobility and mode of nutrient absorption ability. This traditional idea of classification prevailed almost until the middle of the XIX century when new approaches (three Kingdom system by Haeckel [3], two Empire system by Chatton [4], four Kingdom system by Copeland [5], were established, but non of them have recognized fungi as a separate group.

Progress in electron microscopy and biochemical techniques has highlighted important differences between living organisms, distinguishing fungi as organisms substancially different from others in nutrition, by apsorption, cell organization and structure, storage compounds, haploid nuclei, photomorphogenesis, hormonal system, etc. In classification proposed by Whittaker in 1969, fungal organisms have finally gained the position they deserved – the position of separate Kingdom Fungi in Five Kingdom system including Monera, Protista, Planta, Fungi and Animalia according to their multicellular cell organisation and living style [6].

New revolution in biological classification began with the use of molecular phylogenetic analyses in the 1970s, based primarily on the ribosomal RNA (rRNA) genes which are highly preserved and present in all organisms containing enough information. Comparison of small rRNA subunit, done by Carl Woese, demonstrated that there are three evolutionary diverse groups of organisms, named domains, two prokaryotic – Bacteria and Archaea, and one

eukaryotic – Eucarya [7, 8]. In this phylogenetic tree, plant, fungal and animal kingdoms form a cluster at the top of the Eucarya Domain and are often termed as «crown» eukaryotes [9]. Analyses of rDNA and protein-coding genes proved that fungi are more closely related to animals, being their closest relatives, than to plants ([10], [11], [12]). In the last three decades, molecular phylogenetic studies provided a better understanding of fungal diversity and caused constant changes inside the fungal tree of life. It has been demonstrated that several groups of organisms traditionaly classified and studied as fungi are acctualy outside of this group [13]. Slime molds, previously classified as a phylum Myxomycota inside the Kingdom Fungi, were proved to belong to the kingdom Protozoa/Amebozoa [14], devided into four phyla [9]. Oomycota, Hyphochytridiomycota and Labyrinthulomycota have been moved to the kingdom Straminipila [15] / Chromista ([16], [17], [18]). In contrast to these findings, certain organisms which have earlier been placed in other eukaryotic groups have, for the first time, found themselves inside the Kingdom Fungi. Some examples are: *Pneumocystis* – pathogen, once classified as a protozoan, now the member of the Taphrinomycotina in the Ascomycota ([19], [20], [21]), *Hyaloraphidium* - thought to be algal genus, now in the fungal phylum Chytridiomycota [21], Microsporidia – for a long time considered as a most primitive, early divergent eukaryotic group classified as a specific protozoan phylum, now recognized as a highly specialized and reduced fungi, included in the kingdom Fungi or at least concerned as a sister group ([22], [23], [24], [25]).

Four large groups which have been traditionally recognized as the «true fungi»: Chytridiomycota, Zygomycota, Ascomycota and Basidiomycota, also suffered dramatic changes regarding molecular phylogeny. In the 2001, arbuscular mycorrhizal fungi previously known as the order Glomerales/Glomales, which are primarly filamentous and lack flagella, were excluded from the Zygomycota and recognised as a unique phylum Glomeromycota [26]. According to phylogenetic studies previously reported, Ascomycota and Basidiomycota have been proven to represent a monophyletic clade and sister taxa. The clade containing these two taxa is now classified as a subkingdom Dikarya [27], although earlier was recognised as Dicaryomycota [28]. As it was demonstrated by the analysis of rDNA, Glomeromycota is a sister clade with the Dikarya ([13], [26], [29], [30]) forming a clade labeled as «Symbiomycota» (since most of the members form symbioses) [30]. Traditional Chytridiomycota and Zygomycota represent a basal fungal phyla, with earliest divergence, that have been long known as polyphyletic and paraphyletic ([13], [31]).

Molecular phylogenetic studies have brougth us priceless opportunity to take a deeper look into the tree of life and better understanding of relations among living beings. When fungi are concerned, these techniques showed us that we can not rely completelly on morphological traits and that many of an undiscovered species and phylogenetic relations lie hidden in the world of genes. In the recent classification of Hibbett *et al.*, based on the monophyly supported by the number of published molecular phylogenetic studies, new changes have been proposed for many of the basal fungal lineages [32]. The Chytridiomycota is retained as the phylum, containing two classes: Chytridiomycetes and Monoblepharidomycetes. The orders Blastocladiales and Neocallimastigales have been raised to the level of phylum: Blastocladiomycota (already in James *et al.* [33]) and Neocallimastigomycota. The Zygomycota is not accepted as the phylum and it´s former members are distributed among the phylum Glomeromycota and four subphyla *incertae sedis*: Mucoromycotina, Kickxellomycotina, Zoopagomycotina and Entomophtoromycotina [32].

The contemporary researches based on molecular phylogenetic analyses recognized fungal kingdom as one of five eukaryotic kingdoms containing seven phyla: Chytridiomycota, Neocallimastigomycota, Blastocladiomycota, Microsporidia, Glomeromycota, Ascomycota, Basidiomycota (Figure 1). Subkingdom Dikarya contains only two phyla: Ascomycota and Basidiomycota, representing monophyletic clade of "crown fungi" which are recognized as macro-fungi due to clearly visible fruiting-bodies. As opposed to these, micro-fungi comprise the microscopic organisms, yeasts and molds, that are commonly recognized as producers of toxic substances.

Organisms that we used to know as Fungi Imperfecti – Deuteromycetes – Mitosporic fungi, are now also being classified thanks to the molecular studies. Many of them already found their place inside diferent phyla while the rest of them are still waiting to be classified.

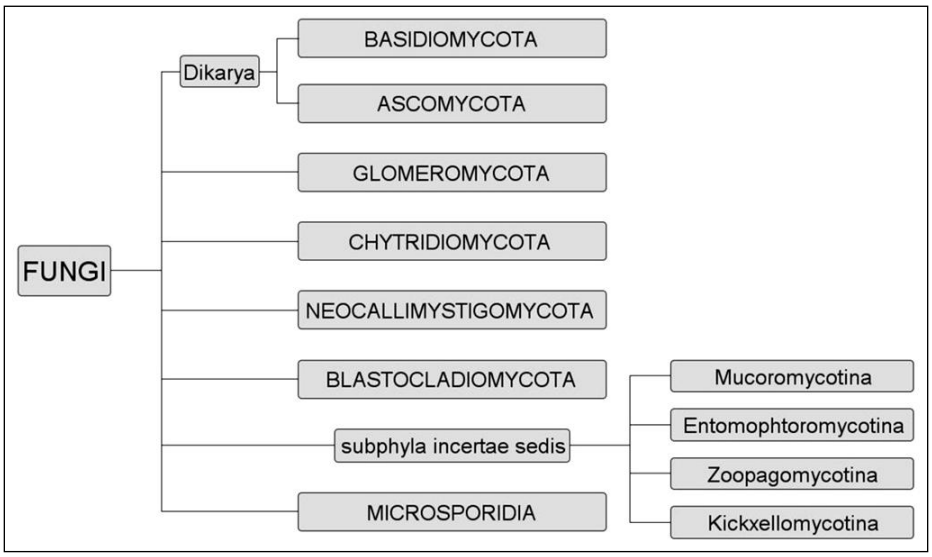

Figure 1. Seven phyla of Fungal Kingdom based on Hibbet et al., 2001[32].

1.2. Mycological Terms

Here are briefly explained some of mycological terms frequently used in this chapter. Macrofungi is the term usualy used for a macroscopical fungi, visible to the naked eye, which form a large fruiting bodies (members of the phyla Ascomycota and Basidiomycota, although a few are Zygomycota). Macrofungi with a morphologically different kind of the fruiting bodies are called pufballs, stinkhornes, bird's nests, morels, earth stars, truffles, coral fungi, etc. Macrofungi can be terrestrial (saprobes or mycorrhizal symbonts) or pathogens of plants, animals and fungi. Mushroom is a fleshy fruiting body of a different Basidiomycota, which is produced above ground (epigeous) and consists of a stem and a cap, with gills or pores underneath the cap. Fruiting body (also known as sporocarp, carpophore or fruit body) is a spore-bearing structure, rising from a substratum and representing part of the sexual phase of a fungal life cycle. Sporocarp of the Basidiomycota is also named basidiocarp and of the Ascomycota – ascocarp. On the other hand, mycelium (plural: mycelia) is a vegetative part of the fungus, growing inside the substratum and consisting of hyphae. Hypha is the basic

structural unit of filamantous fungi, in the form of cylindrical, thread-like, apical growing structure which can be divided into compartments containing septum (pl. septa) or coenocytical (without septa). Terricolous fungi are the fungi growing on, or under the soil surface. Saproxylic fungi are the one growing on the wood substratum, while the term lignicolous (or sometimes xylophagous) is refered particulary to the fungi which have ability to decompose wood (wood-decaying). Fungi fruiting on woody substrata are usually either saprobes (degrading dead organic matter) or plant pathogens (using live organic matter).

1.3. Nutritional Groups

Generally, fungi belong to a group of *heterotrophic organisms*, with few exceptions of their ability to supply themselves chemotrophically by inorganic carbon, which resulted in their adaptation to the use of different organic substrates. There are three general nutritional groups of fungi: saprotrophic or saprobic, which grow on a substrate formed after death of organisms (term saprophytic has been replaced recently by terms saprotrophic or saprobic since they do not belong to plants), parasites, which attack living organisms and mutualistic fungi, which form associations of a mutual benefit with a variety of organisms.

Most fungi live as saprotrophs obtaining their nutrients from dead organic matter such as wood, leaf litter, soil, dung, dead animal and fungi causing catabolic dissimilation processes, thanks to extracellular digestion enzymes which are secreted by mycelia. In that way nutrients that are locked-up in the form of complex organic compounds release thus making them useable by other living organisms. This is making fungi as vital components of healthy natural ecosystems, especially forests. Deacon (2006), [9] emphasizes that saprotrophic fungi are distinguished in their behavior and capability to degrade certain types of substrates, as a consequence of colonising the same resource of nutrients in a different time and sometimes are also overlapped. Pioneer species use simple soluble substrates and usually cannot degrade the complex structural polymers. They are good competitors, characterized by rapid growth and short life cycle. Polymer-degrading fungi, which are substrate-specific, colonize and decompose the major structural polymers of hosts (such as cellulose, hemicellulose, chitin). They have an extended growth phase and are able to defend a food source via antibiosis or by taking away mineral nutrients from the substrate. Some fungi are specialized for the degradation of resistant organic materials (lignin, suberin, keratin, etc.) thereby achieving access to polymers. They are capable of defending the substrate by antagonism or inhibition, and have a mechanism to obtain mineral nutrients that were utilized by previous colonizers.

Among parasitic fungi, three major groups are recognized: 1 - obligate parasitic fungi, 2 - parasites of weakness and 3 - facultative parasitic fungi. Some parasites have very specific host requirements and may only attack a single species, while others may parasitize a variety of hosts genera [34]. Obligate parasitic fungi, also known as plant, animal and human pathogens, attack living organisms causing tissue damage and diseases that can lead to death of the host organism. Opposite to them, parasites of weakness are not able to attack healthy organism but require host weakness for its development. They colonize initially damaged, weakened or immuno-compromised host organisms. Those fungi that can infect host only on the places of damaged tissue, such as the surface of a wound, are often reffered to as a wound parasites. Facultative parasitic fungi are the ones that after death of the host keep on using the same tissues but now as a saprotrophs. It means that they are also capable of using dead

organic substrate as the carbon source. In the nature, it can often be difficult to determine clear boundaries among these sub-categories [35].

Third nutritional group comprises fungi that form mutualistic associations with plants, animals and prokaryotes. Some of the best known beneficial associations in wich fungi are involved are lichens, with algae as a partners, and mycorrhizae, essential relationships with the roots of almost all living plants. In lichens, fungi protect algae from external effects and provide with water and mineral nutrients while algae supply fungi by organic nutrients. In mycorrhizal association, the plant provides the fungus with water and organic compounds, while the fungus provides the plant with the scarce minerals such as phosphorous [36]. Mycorrhizal fungi can protect plants against pathogenic fungi and microorganisms, as well as from the harmful effects of heavy metals and radionuclids. They do not grow without their host and many of them are host-specific, growing with one kind of plant.

Interesting mutualistic relationships occure between fungi and animals. Some ants and termites cultivate fungi in a special gardens“ (preparing a substratum for fungal growth and introducing them to it) which serve as a food source for their larvae. Certain wood beatles and wasps also inoculate tunnels created in the wood with fungi wich they farm for food. Gut sistem of ruminants (such as moose, cow, etc.) contains community of microorganisms, including fungi, that help these animals to digest cellulose [37]. Fungivorous animals, especially those that feed on a hypogeous fungi, have a significant role in a long distance spore dispersal and there we have another example of the animal/fungus mutualism (for many species of a small mammals fungi are a dominant food) [38]. All of these exemples proved the ubiquity of fungi in nature as well as their importance for the functioning of other organisms.

2. Wood-Decaying Fungi

Doubtless, fungi represent an exceptionally significant component in nature since they are the main agents that cause the decomposition of organic matter in terrestrial and aquatic ecosystems. One of their most important roles is degradation of wood, since they are the only organisms that can completely degrade all of its components [39]. Lignicolous fungi inhabit substrata that differ in size (tiny twigs, huge trunks), state of decay and moisture content [40]. Their decay activities are conected to the recycling of lignocellulosic and mineral nutrients back into the ecosystem but also on maintenance of mycorrhizal fungi in seasonnally dry forests since woody debris acts as a moisture sink. Moreover, many ectomycorrhizal fungi are climbing up on wood when fruiting and simultanously may obtain nutrients from the wood by degrading organic compounds.

In addition, wood presents the major storehouse of the carbon fixed by photosynthesis in the Biosphere, making wood-decaying fungi essential agents in the biogeochemical cycling of carbon in nature. Wood material is mostly made of fibrous and soft, amorphous cellulose and hemicellulose imbedded in glue-like, hard-to-degrade lignin (it is chemically complex, variable, nonhydrolysable and water-insoluble), building complex lignocellulose substrates which are unavailable to most saprotrophic organisms. Another obstacles for the wood-decomposers are very low content of available nitrogen (ratio C : N = 500 : 1), low content of phosphorus and toxic compounds concentrated in the heartwood [9]. Most efficient wood-

decaying fungi are found in the phylla Basidiomycota and Ascomycota. Many of them are highly specialized in the sense of tree species or decomposition stage. There are a number of classifications of wood-degrading fungi according to different criteria, and categorization given here is based on a way of attacks on lignocellulose complex, i.e. according to the primary enzyme activity: 1 - white-rot fungi, 2 - brown-rot fungi, and 3 - soft-rot fungi.

2.1. White-Rot Fungi and Lignolitic Enzymes (Ligninases)

White-rot basidiomycetous fungi are the only organisms known to mineralise both lignin and carbohydrate components of wood to carbon dioxide and water due to posessing the specific enzymes for extracellular oxidation and depolimerisation of lignin [41].

The term white-rot fungi is derived from bleached residue of cellulose after complete lignin degradation and to a half extent the degradation of hemicellulose and cellulose. Contrary, the brown-rot fungi merely modify lignin while removing carbohydrates in wood [39]. Enzymatic „combustion“, a process wherein enzymes generate reactive intemediates, whithout directly control the reactions of lignin breakdown has been proposed as the mechanism of lignin biodegradation [42]. Wood decay and the biogeochemical cycling are the final consequences of lignin biodegradation.

Lignin is an aromatic polymer forming up to 30% of woody plant tissues providing rigidity and resistance to biological attack [42]. It is the most abundant renewable aromatic polymer on the Earth, composed of non-phenolic (80-90%) and phenolic structures [43]. It has been shown that fungi degrade lignin by secreting enzymes collectively termed “ligninases”. Its structure is complex and the process of its biodegradation is based on specific machanism of few lignolytic (ligninolytic) enzymes: laccases, manganese dependent peroxidases – MnP, lignin peroxidases-LiP, and non-specific strong oxidants able to destabilise and fragment lignin [41]. These include two lignolytic families: 1) phenol oxidase (laccase) and 2) peroxidases (lignin peroxidase (LiP) and manganese-dependent peroxidase (MnP)) [44]. This enzyme system is extracellular, relatively non-specific and cause generation of enzymatic and non enzymatic oxidants. The activity of these enzymes are influeneced by pH, temperature, substrate concentration, the pesence of mediators and vertryl alcohol, cofactors (Cu^{2+}, Mn^{2+}) inhibitors, and organic acids (e.g. citric, oxalic, and tartaric). White-rot basidiomycetes such as *P. chrysosporium* and *T. versicolor* ([45], [43]) have been found to be the most efficient lignin-degrading microorganisms studied.

Lignin peroxidase (LiP) called ligninase is the enzyme, heme-dependent-peroxidase with an unusually high redox potential and low optimum pH. It is able to oxidize metoxylated aromatic rings without a free phenolic group, generating cation radicals that can react further by variety of pathways. Hence, LiP is able to oxidize the non-phenolic part of lignin, but it was not detected in many lignin-degrading fungi. In addition, it has been widely accepted that the oxidative lignolytic enzymes are not able to penetrate the cell walls due to their size. Thus, it has been suggested that prior to the enzymatic attack, low-molecular weight diffusible reactive oxidative compounds have to initiate changes to the lignin structure ([46], [47]).

Manganese peroxidase (MnP) is also a heme-peroxidase that shows stronger preference for Mn(II) as its reducing substrate. It oxidise only phenolic substrates and the product Mn(III) forms a complex with organic acids and diffuses away to oxidize other materials,

such as lignin. It has lower redox potential then LiP and it does not oxidize nonphenolic lignin models [42].

Laccase is a copper-containing oxidase and it does not require peroxide. Similarly to Mn-peroxidase it oxydizes only those lignin model compounds with a free phenolic group, forming phenoxy radicals.

Cellobiose is the heme-flavin enzyme that oxidizes cellobiose and some other carbohydrates and reduces quinones and the radicals produced by the action of lignin peroxidase, Mn-peroxidase and lacase on lignin model compounds.

The activity of these enzymes depends on a supply of H_2O_2 from glucose-1-oxidase, glucose-2-oxidase, glyoxyl oxidase, aryl alcohol oxidase, and methanol oxidase. Intracellular metabolism also supplies oxalate and other organic acids which can chelate Mn(III). It is clear that the lignin myco-degradation is extremely dependent on the supply of oxygen and therefore does not take place in a water-saturated conditions. Also degradation of lignin can be potentially dangerous for the fungus because some intermediates of this process can be mycotoxic. White-rot fungi produce such compounds but they can detoxify them by polymerizing it into pigments similar to melanin [41].

The most important white-rot fungi are the two main tree pathogens: *Armillaria mellea* and *Heterobasidion annosum*, and many saprotrophic mushrooms, including stump colonizers such as *Coriolus versicolor*, *Xylaria hypoxylon* and *Xylaria polymorpha* which degrade all components of wood including lignin. However, they have distinctive and very strong ability of nitrogen supply and also have been shown to recycle effectively nitrogen within their mycelium [48].

2.2. Brown-Rot Fungi

Only aproximatelly 6% of the wood-rotting fungi are brown-rots [49]. They are capable of cellulose and hemicellolose degradation but leave the lignin more or less intact in the form of brown structure on the wood surface, often cracked and appearing as a stacked bricks. Mechanism of cellulose and hemicellulose degradation is based on extracellular production of hyghly reactive hydroxyl radical (OH·radicals) that are produced through Fenton reactions to start degradation. Many of fungi such are *Laetiporus sulphureus* are excreting high levels of oxalic acid, hydroquinones and glycopeptides that would serve for binding and directly reducing Fe^{3+} to Fe^{2+} to provide reactants for subsequent hydroxyl radical formation [50]. Brown-rot decay almost exclusively occurs in terrestrial systems, with the greatest diversity and impact taking place in temperate and tropical zones [51].

Brown-rot fungi are predominantly Basidimycota, mostly confined to nine families (Polyporaceae, Auriculariaceae, Coniophoraceae, Sparassidaceae, Corticiaceae, Stereaceae, Tricholomataceae, Coprinaceae and Paxilaceae) and more commonly associated with conifer forests. Representatives such as mushroom *Schizophyllum commune*, *Fomes fomentarius*, *Daedalea quercina* and *Piptoporus betulinus* form a macroscopic, sometimes rather large console-shaped fruit bodies on a dead tree trunks ([52], [53], [54], [55]). Interesting member of the „brown-rot fungi“ group is a so-called „dry-rot“ fungus, e.g. *Serpula lacrymans*. It causes the same type of wood decay as the other members of the „brown-rot“ group, but the term „dry-rot“ derives from the fact that this cellulose degrading fungus, during the process,

can produce a sufficient amount of moisture that allows unimpeded growth even in extremely dry conditions [9].

2.3. Soft–Rot Fungi

Soft rot causing fungi belong to the phylum Ascomycota and the group of mitosporic fungi. Comparing to white-rot and brown-rot fungi, they are not agressive decay organisms and may not be good competitors in normal conditions. Thus, they are usually found in damp environments and woods with limited access to oxygen [51]. Soft-rot fungi decompose cellulose and hemicellolose, with little or no effect on lignin, producing typical chains of cavities inside the cell wall. Most of them require high levels of nitrogen, which they draw from the wood, if available, or the environment such as soil and water [9].

3. Enzymology of Wood Degradation

Cellulolytic complex of deuteromycetes species of the genera *Trichoderma* (*T. harzianum*, *T. viride*, *T. reesei* and *T. koningii*) ([56], [57], [58], [59], [60], [61]) and *Gliocladium* (*G. virens*, *G. roseum*) ([61], [62], [63]) and anamorphs of Ascomycotina (*Chaetomium erraticum*, *Penicillium funiculosum* and *Fusarium solani*) ([64], [65], [66]), hydrolyze crystalline cellulose applying joint action of enzymes (endo-β-1,4-glucanase, exo-β-1,4-glucanase or celobiohydrolase (celobiase) and β-glucosidase).

Endoglucanase activity produces new shorter chains with accessible chain ends which then are attacked by celobiase. However, since the hydrolysis of glycosidic bonds is potentially reversible reaction, the separation of crystalline regions from cellulose chains will be prevented or slowed down due to the limitations caused by intramolecular and intermolecular hydrogen bonds. Therefore, these two enzymes must act sequentionally as well as successively, quickly one after the other, to prevent re-formation of glycosidic bonds.

Lignocellulose degradation by fungi is performed by complex mixtures of cellulases [67], hemicellulases [68] and ligninases ([69], [70]), reflecting the complexity of the materials. Cellulases and most hemicellulases belong to a group of enzymes known as glycoside hydrolases. Currently more than 2500 glycoside hydrolases have been identified and classified into 115 families [71]. Fungal cellulases (hydrolysis of β-1,4-glycosidic bonds) have been mostly found within a 11 glycoside hydrolases families ([71], [72]).

3.1. Fungal Extracellular Cellulases

Hydrolysis of the β-1,4-glycosidic bonds in cellulose can be achieved by many different enzymes known as cellulases which use two different catalytic mechanisms, the retaining and the inverting mechanisms ([67], [72]). Many different fungal species have the ability to degrade cellulose by producing extracellular fungal cellulose-degrading enzymes including endo-cleaving (endoglucanases) and exo-cleaving (cellobiohydrolases). Endoglucanases can hydrolyze glycosidic bonds internally, inside the cellulose chains, whereas cellobiohydrolases

act preferentially on chain ends. The products of these enzymatic reactions are mostly a disaccharide cellobiose and, to a lesser extent, cello-oligosaccharides, which will be further hydrolyzed by the third group of enzymes - β-glucosidases [73]. Cellulases mostly have a small independently folded carbohydrate binding module responsible for binding the enzyme to the crystalline cellulose and thus enhance the enzyme activity [67]. Currently, many carbohydrate binding modules have been identified and classified into 54 families, however only 20 families have been found in fungi [74]. Endoglucanases (EGs), endo-1,4-β-glucanases (EC 3.2.1.4, endocellulase) also referred to as carboxymethylcellulases (CMCase), are named after the artificial substrate used to measure the enzyme activity. EGs initiate cellulose breakdown by attacking the amorphous regions of the cellulose, making it more accessible for cellobiohydrolases by providing new free chain ends. This has been shown by the effect of the enzyme on carboxymethyl-cellulose and amorphous cellulose [75]. For the fungal EGs, optimal pH ranges mostly between 4.0 and 5.0 and optimal temperature is from 50 to 70 °C. Many fungi produce multiple EGs, for example, *Trichoderma reesei* produces at least 5 EGs whereas three EGs were isolated from white-rot fungus *Phanerochaete chrysosporium* ([76], [77]). In addition, some EGs lack a carbohydrate binding module while some other EGs with carbohydrate binding module have been described. Cellobiohydrolases (CBHs) EC 3.3.1.91, exocellulase) preferentially hydrolyze β-1,4-glycosidic bonds from chain ends, producing cellobiose as the main product. CBHs have been shown to create a substrate-binding tunnel with their extended loops which surround the cellulose ([78], [79]). Similar to EGs, CBHs are monomers with no or low glycosylation with optimal pH between 4.0 and 5.0, but the optimal temperatures are wider, from 37 to 60 °C. Some CBHs can act from the non-reducing ends and others from the reducing ends of the cellulosic chains, which increases the synergy between opposite-acting enzymes. Cellobiose, the end product of CBHs, acts as a competitive inhibitor, which can limit the ability of the enzymes to degrade all of cellulose molecules in a system ([76], [78]). From many filamentous species such as *T. reesei*, *T. harzianum*, *G. virens* ([57] [63]) and basidiomycetes such as white-rot and brown-rot fungi β-glucosidases (BGLs) have been isolated (EC 3.2.1.21). By using the retaining mechanism, β-glucosidases hydrolyze soluble cellobiose and cellodextrins (attacking β-1,4-glycosidic bonds) to glucose, and are thus competitively inhibited by glucose [80]. BGLs show the highest variability among the cellulolytic enzymes due to their structure and localization. While some BGLs have a simple monomeric structure with around 35 kDa molecular mass (e.g. *Pleurotus ostreatus*) [81], some others have dimeric (e.g. *Sporobolomyces singularis* with 146 kDa) [82] or even trimeric structures with over 450 kDa, e.g. *Pisolithus tinctorius* [83]. In addition, most of BGLs are glycosylated and in some cases, such as the 300 kDa monomeric BGL from *Trametes versicolor*, the glycosylation degree is up to 90% [84]. Regarding localization, BGLs can be grouped into three different types including intracellular, cell wall-associated and extracellular [85]. Optimum pH for the enzymes relies on enzyme localization while the optimum temperature ranges from 45 to 75 °C.

3.2. Fungal Hemicellulases

Several different enzymes are needed to hydrolyze hemicellulose, due to its heterogeneity [86]. Xylan is the most abundant component of hemicellulose contributing over 70% of its

structure. Xylanases are able to hydrolyze β-1,4 linkages in xylan and produce oligomers which can be further hydrolyzed into xylose by β-xylosidase ([57], [58]). Not surprisingly, additional enzymes such as β-mannanases, arabino-furanosidases or α-L-arabinanases are needed, depending on the hemicellulose composition which can be mannan-based or arabinofuranosyl-containing [87]. Similar to cellulases, hemicellulases are usually modular proteins and have other functional modules, such as carbohydrate binding modules, in addition to their catalytic domains. Also similarly to cellulases, most of the hemicellulases are glycoside hydrolases, although some hemicellulases belong to carbohydrate esterases which hydrolyze ester linkages of acetate or ferulic acid side groups ([87], [88]). Hemicellulases belong to 20 different glycoside hydrolases families and all of them, except 4, 8, 52 and 57 families, have been found in fungi. Similarly to cellulases, aerobic fungi such as *Trichoderma* and *Aspergillus* secrete a wide variety of hemicellulases in high concentrations and these work in a synergistic manner ([87], [74]).

3.3. Fungal Mechanisms of Oxidative (Non-Enzymatic) Lignocellulose Degradation

A few decades ago, non-enzymatic mechanisms for plant cell wall polysaccharide degradation were also considered and over the time more evidence for these was found. The non-enzymatic degradation mechanism is mostly assisted by oxidation through the production of free hydroxyl radicals (OH·). In fact, many white-rot and brown-rot fungi have been shown to produce hydrogen peroxide (H_2O_2) which enters the Fenton reaction, resulting in release of OH· ([89], [90]). These free radicals attack polysaccharides as well as lignin in plant cell walls in a nonspecific manner providing some cleavages which make it easier for the lignocellulolytic enzymes to penetrate ([91], [43]). Three different pathways have been found for the generation of free radicals including cellobiose dehydrogenase (CDH) catalyzed reactions, low molecular weight peptides/quinone redox cycling and glycopeptide-catalyzed Fenton reactions [76]. CDH, an extracellular monomeric protein with some glycosylation has been identified in a number of wood-degrading and cellulose-decomposing fungi including basidiomycetes (mostly white-rot fungi) and ascomycetes growing on cellulose. The enzyme is able to oxidize cellobiose, higher cellodextrins and other disaccharides or oligosaccharides with β-1,4 linkages. In addition, CDH with (in Ascomycetes) or without (in Basidiomycetes) carbohydrate binding modules has been identified since, even in the absence of carbohydrate binding modules, they are able to bind to cellulose through hydrophobic interactions [92]. CDH production is higher due to cellulases and hemicellulases activity ([93], [94]). It is now widely accepted that CDH is able to degrade and modify all three major components of the lignocellulosic residues (cellulose, hemicelluloses and lignin) by producing free OH· in a Fenton-type reaction [76]. It was also found that white-rot and brown-rot fungi produce low molecular weight chelators which are able to penetrate into the cell wall. For example, *Gloeophyllum trabeum* produces a low molecular weight peptide (known as short fiber generating factor, SFGF) which can degrade cellulose into short fibers by an oxidative reaction ([43], [95]). It has also been reported that some of these low molecular weight compounds are quinones which have to be converted to hydroquinones by some fungal enzymes and then through Fenton reaction, free hydroxyl radicals will be produced ([43], [74]). Different glycopeptides with different molecular weight (ranging from 1.5 to 12 kDa)

have been found in many brown-rot fungi such as *G. trabeum* [96] and white-rot fungi such as *P. chrysosporium* ([47], [97]). Similar to the other mechanisms, glycopeptides are able to catalyze redox reactions and thus produce free hydroxyl radicals.

4. Biotechnological Application of Lignolytic Enzymes

There are many possible applications based on specific enzyme system of white-rot fungi to serve humans and it is resonable to expect that their range will continue to expand. Environmental conditions have an important influence on the synthesis and activities of lignolytic enzymes in conversion of natural and agricultural biomass or enzymatic conversion of pollutants and aroma-chemical precursors. In addition, enzymology and physiology of lignin catabolism of white-rot fungi are also a suprime tool in controlling synthesis and degradation of structurally similar organic compounds. The ability to produce various types of peroxidases and laccases, in addition to H_2O_2 and hydroxyl radicals is considered the initiating key to the degradation of many types of complex compounds by the white-rot fungi [50]. Considerable potential of lignolytic enzymes could be employed in a number of biotechnological applications.

4.1. Biopulping, Biobleaching and Decolorisation of Industrail Efluents

Biopulping is the directed treatment of plant biomass with lygnolytic microorganisms and enzymes prior to chemical and mechanical pulping to obtain a product enriched in polysaccharides and cellulose in particular. This process involving white-rot fungi is dependent on different factors such as biochemical caracteristics, fungal strain and substrate, culture conditions and incubation time [41]. Some efforts has been made toward developing cell free biopulping process that use isolated lignolytic enzymes which was overlapped because of the difficulties in costs and difficulties of generating and maintaining optimum conditons for enzyme activity. Biobleaching is the biological removal or destruction of smaller quantities of residual lignin and other coloring matter that remain after process of pulping. MnP and laccase have been shown to possess these abilities. White-rot fungi may have some applications in remediation of industrial wastewaters by decolorising the water and degrading toxic compounds. Effluent water may contain underivatised lignin, lignin derivatives, lignosulphate, tannins, phenolics and other colored and toxic compounds. Strain of *Lentinus edodes* was proved to remove 73% of the color in 5 days and olive waste water was reduced in color for 45%, total organic carbon by 75% and total phenols by 60% within 4 days [98]

4.2. Biosorption

Process when microbial biomass (dead or living) is used to remove metals from solutions is denoted as biosorption. In the past few years macrofungi have appeared as potential agents

for the remediation of wastewater containing toxic metal ions. Fungal filamentous or hyphen structure features are very important in the effective substrate colonization, with hyphen that have apical growth and production of high amount of lateral branches and complex fruiting bodies. Living cells accumulate metals in two phases: physical–chemical reaction e.g. adsorption, intracellular uptake through the plasmalemma or simply membrane adsorption and extracellular precipitation in or around the cell wall [99]. Dead cells can only adsorb metals on the cell walls. Accumulation of some radionuclides (actinides) is prevalently a consequence of adsorption while for caesium it is a characteristic process of intracellular uptake.

In a recent study, adsorption potential of *P. ostreatus* showed that this species is an efficient biosorbent because of fast metal removal rate, remarkable biosorption capacity for Ni, Cr, Cu and Zn and high regeneration ability [100]. Wood-inhabiting basidomycets are an useful source of mycelial biomass for biosorption of metal ions due to ease of cultivation, high yield and non-hazardous nature. The most important role in white-rot fungi is dedicated to polysaccharides, proteins or pigments that have a good capacity for heavy metals binding [101]. The adsorption of heavy metals to the mycelia of white-rot fungi fits the Langmuir and Freundlich adsoption isoterm ([101], [100]).

Mycelia of four white-rot fungi, *D. quercina*, *G. applanatum*, *Stereum hirsutum* and *Schizophyllum commune* cultured in a liquid media showed that the preference for specific heavy metal is species-specific and differrent when a mixture of metals is offered. Pb content was maximal in *S. hirstum*, while *G. applanataum* contained maximal values of Cd, Al and Ca [102].

4.3. Xenobiotic Compound Degradation (Mycoremediation)

Major classes of resistant and environmentally toxic or hazardous substances are chlorinated organics, polycyclic aromatic hydrocarbons (PAHs), nitrated organics and textile dyes [41]. White-rot fungi have attracted the highest interest due to their ability to degrade simultaneously large variety of chemicals. Polychlorinated biphenyls (PCBs) are degraded by *Pleurotus ostreatus* and *Trametes versicolor* strains ([103], [104]). Recent study reporeted that *G. lucidum* is a promising white-rot fungus to degrade PAHs such as phenantrene and pyrene in the environment [105].

Lignolytic enzymes can transform chemical pollutants that are relatively long-lived in the environment due to its high molecular weight and low (aqueous) insolubility. They can cause biotransformation of a group of lipophilic pollutants that are generated during incomplete combustion of organic carbons such are fossil fuels, wood and municipal solid waste ([106], [107], [108]) or industrial effluents into benign or less harmfull products. This is the most attractive property that can be used in processess of biodegradation and biodeterioration of soils, sediments or water where PAHs are accumulated. PAHs are also componenets of wood preservatives. Species of brown-rot fungi such are *Fomitopsis*, *Lentinus* and *Laetiporus sulphureus* are the most studied fungi able to degrade xenobiotic compounds, although specific mechanisms are still not well characterized [50].

In comparative studies on lignin and PAHs degradation using 130 wild basidiomycetous fungi, *Phellinus* species showed better degradation in solid state fermentation as the most efficient strain. According to different predominant enzyme activity of manganese-dependant

peroxidase (MnP) in *Phellinus* sp., contrary to *Laetiporus sulphureus* where lignin peroxidase was predominant, the route of PAHs degradation was species specific [108]. The species *Lentinus (Panus) tigrinus* showed high PAHs degradation rate being more pronounced than in *Irpex lacteus* [107]. Moreover, a preliminary assessment of strain/carrier combination is fundamental prior to field-scale mycoremediation.

4.4. Enhacement of Digestibility of Ruminant Feed and Production of Edible Fungi

Animal feed can be upgraded with white-rot fungi by enhancing digestibility of polysaccahrides from agricultural or forest residues containing lignin. Biological delignification of animal feed will be predominately established in the future in concern with the production of edible fungi [42]. White-rot basidiomycets, e.g. *Pleurotus ostreatus*, are actively involved in the re-circulation of carbon at a global level, since being lignin-degrading ones. Ability of white-rot fungi to use waste lignocellulosic material, e.g. agricultural waste (wheat straw, bagasse, coffee pulps, etc.) and brown coal that are abundant, cheap and non utilitarian, can be used for production of food for humans.

Some lignin degrading fungi produce edible sporocarps and can directly convert lignocellulose into food for humans. Fruiting bodies of *Lentinula edodes*, *Auricularia polytricha*, *Pleurotus ostreatus* and *Flammulina velutipes* are functional food, comprising both food and medicine. Edible mushrooms represent high value food, which are used for human nutrition because of their excellent flavour, texture, and can be eaten fresh or used in dry form for additives or for making healthy beverages. They are mostly used in a form of dietary supplements from Asian to Europe and North America.

At the same time macrofungi give the opportunity to the mankind in resolving stress problems. Recently, different genera of basidiomycetous fungi have been used as sources of natural bioactive metabolites of pharmacological interest, with various medical effects: antitumor, immunomodulating, cardiovascular, antimicrobial, antiparasitic, antidiabetic, hepatoprotective, etc. Biological activity and chemical composition of fungal species varies broadly and depends on strain and substrate and mode of growing. With regard to the fact that production of oxygen radicals provokes pathological changes in organisms, antioxidants derived from these edible and medicinal fungi could diminish pathological disorders in humans [109], [110], [111], [112].

Many fungal bioactive compounds (extracellular and intracellular), as products of primary or secondary metabolism, can be brought in human use both as novel medicines e.g. antibiotics [113], cholesterol-lowering agents [114], immunosuppressive drugs [115], agents against insects [116] and microorganisms [117], as well as for improvement of the antioxidant status during aging ([118], [119]). It is assumed that natural compounds, being part of the ecosystem, might be more compatible and less toxic to humans and the environment. Basidiomycetous macrofungi presently produce about 5% of all antibiotics, but there are studies showing that up to 40% of them are able to produce such medically important substances. According to these investigations their interaction with environmental components, especially with pollutants, attracted the attention of scientific public worldwide [120].

World market have been increasing over past decades ([121], [122]). *Ganoderma* species are the most investigated white-rot mushrooms for its pharamaceutical applications [123]. It can be harvested on timber logs, tree stumps, expanding cultivation of these edible and medicinal species in the future. Cultivation of *L. edodes* is usually on chestnut and oak, while *P. ostreatus* is grown on poplar originating substrate. Inoculation of logs is done by drilling holes in the timber and insertation dowels infected with fungal mycelium or insertation of cultures grown on sawdust. Fruiting depends on appropriate reduced temperature and carbon dioxide concentration. It should be noted that sterilised substrate is necessary tool for the cultivation, as well as reliable control of insect pests and parasitic fungi without resorting to pesticides.

5. Relations between Polluted Environment and Fungi

5.1. Interactions of Heavy Metals with Fungi, Especially White-Rot Macrofungi

Like some other microbial groups (e.g. lichens) macrofungi can accumulate metals from their environment by means of the physico-chemical and biological mechanisms [99]. Unlike lichens that are good bio-accumulators (bio-indicators) of atmospheric pollution ([124], [125], [126]), filamentous fungi are one of best accumulators of metal ions from soil, due to the biological properties of their vegetative mycelia, which are closly associated with the host roots and soil. Mycelium, living in the soil for several months or even years, is a potent absorptive biomass for accumulation of elements, but fungal fruiting bodies show even higher concentrations of these elements.

Mineral nutrients have many roles in fungal life taking part in composition of organic compounds, in activation of specific biochemical paths, etc. Metals are directly or indirectly incorporated in: growth, differentiation, reproduction and the overall metabolic (enzymatic) activities. According to Gadd [99], there is an ultimate chain that connects artificial or natural environment with fungi and metals (Figure 2). Environment affects all life stages of fungi as well as the availability and type of metal. Metal may has positive or negative impact on all life stages of fungi, being dependent on the concentration and type of metal, but also can change physico-chemical characteristics of the environment. Fungi can eliminate metals and organo-metaloids from solutions both by physical and biological processes and by transforming them into other forms. Fungi affect the environment by their metabolic activities and growth.

First studies of the interaction between heavy metals and fungi were organized along with the phytopathological examinations on fungicide preparations, which are based on toxicitie of metals to wood-decaying fungi ([127]). The early preparations of biocides were based on mercury but due to its high toxicity it was superseded by cooper-based word preservatives. Copper-chrom-arsenate (CCA) preservatives have been held in high repute since 1933 but recently their harmful effect was disclosed, giving priority to chromium and arsenic-free wood preservatives. Organotin compounds such as bis-(tri-n-butyl-tin)-oxide (TBTO) and n-

butyl-tin-naphthenate have been used in a wood protection against wood-destroying molds and lower fungi but also against brown-rot fungi [101].

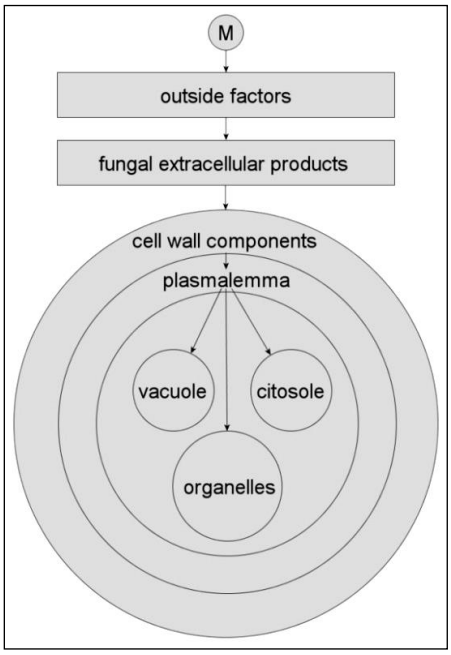

Figure 2. Interactions of heavy metals with fungi.

The environmental problems as well as toxicity tests showing harm of toxic metals, metalloids, radionuclides and organo-metaloids to living resources simulate new phase of investigations involving intake and translocation of metals and radionuclides through the sporocarp of edible fungal species ([128], [129]).

Essential microelements mostly belong to a group of heavy metals which activity is strongly specific, prevalently catalytic when present in very low concentrations. Essential elements for fungi are potassium (K), sodium (Na), magnesium (Mg), calcium (Ca), manganese (Mn), iron, (Fe), copper (Cu), zinc (Zn), cobalt (Co) and nickel (Ni), while unessential are rubidium (Rb), cesium (Cs, aluminium (Al), cadmium (Cd), silver (Ag), aurum (Au), mercury (Hg), lead (Pb), chromium (Cr) etc. Their toxicity relies on species, growth stage, physico-chemical characteristics of metals and environmental factors.

5.2. Environmental Factors and uptake of Metals

The impact of heavy metals on microorganisms depends on the following factors: 1) physico-chemical factors (pH, Eh, anion content, moisture, aeration, content of clay and organic matter), 2) chemical properties and concentration of heavy metal (Hg>Ag>Cu>Cd>Zn>Pb>Cr>Ni>Co); 3) species specific caracteristics and age of microorganism (e.g. age of mycelia and intervals between fructification [130]; 4) temeperature and timing of exposure; 5) chemical composition of microorganisms in a given habitat [131] (Figure 3).

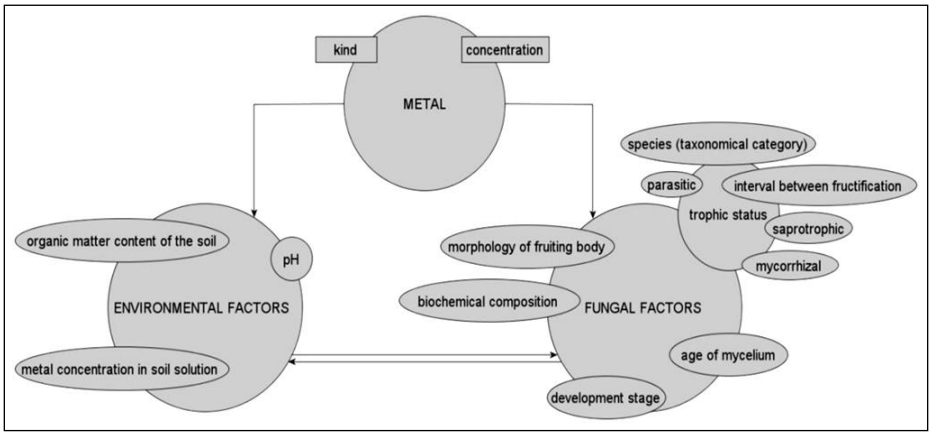

Figure 3. Main factors affecting the accumulation of heavy metals.

The uptake of metals is influenced by metal origin, transport mode (atmospheric deposition or sewage sludge) and biochemical and chemical factors (pH, carrier molecules, etc.) [132].

5.3. Toxicity Mechanisms

Although biotoxicity and bioaccumulation is considered as related to nonessential elements, toxicity of higher concentrations of essential elements is also established (e.g. higher concentrations of Ca^{2+} precipitate phosphate and reduce sporocarp formation) [133]. On the contrary, copper is toxic to the most of fungi even at very low concentration [101]. In a study which analysed the effect of Cu and Zn pollution on the terrestrial fungi in Sweden, most species were affected at intermediate Cu level (600 - 4000µg) while species from *Amanita* genus appeared tolerant to Cu and species belonging to genus *Cortinarius* decreased in abundance near the mill [134].

Wood–rotting fungi are mostly supplied with metals via wood that contains lower concentration and availability of heavy metal ions then soil [55]. Soil is highly influenced by industrial pollution and near motorways or gas-work sites this contamination is often accompanied by the presence of high levels of polycyclic aromatic hydrocarbons PAHs ([107], [55]). Atmospere is the most important transport medium for metals that derived from various sources and due to gravitation aerosol precipitate on vegetation, soil and waters [135]. It has been noticed recently that fruit bodies of white-rot fungi receive significant amounts of heavy metals from the atmosphere ([55], [101]).

Toxic activity of heavy metals is a result of one or more irregular metabolic processes or chemical reactions. Toxicity of heavy metals comprise the following: 1) blocking of functional groups of enzymes and transport systems (e.g. Cd, Pb, Ag, have great affinity towards sulfhydryl groups), 2) removing (by precipitation or chelating) or substitution of essential metal ions from biomolecules or organelles, 3) conformational changes of organic molecules, 4) denaturation and inactivation of enzymes (Ag and Cu have great affinity towards active parts of enzymes, and going through the cell membranes with organic molecules in a form of chelate); 5) degradation of integrity of cell membrane and membrane

of organelles (Ag, U, Au, Cd and Cu, changes membrane permeability). Disruptions of cell membrane is associated with loss of K^+ ions and increase of cell permeability [99] caused by depolarization of electrochemical gradient.

Indirect mechanism of toxicity is connected with induction of free radicals generated in oxydoreductive reactions. In aerobic organisms, the processes of lipid peroxidation is provoked by metals [136] resulting in changing of lipids to peroxy-alkyl radicals and hydroperoxides of fat acids. Complexes with metals that are solubile in lipids can go through the Fenton reaction with hydroperoxides and accelerate this process, what is happening in aqueous solutions of free ions and complexes:

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^- + OH^{\bullet}, O_2^- + Fe^{3+} \longrightarrow O_2 + Fe^{2+}$$

5.4. Effects of Specific Heavy Metals on Fungi

5.4.1. Lead

The lead shows silmilar reactions as Cu and Hg, forming insoluble sulfids, and has affinity for sulfhydryl groups [99]. It causes the damage of plasma membrane and forms a very stable organic chelates. Moreover, the presence of phosphates increases the tolerance of fungi to lead by formation of salts wich are hard to dissolve. Thus, the effects of lead on fungi are not a direct result of toxicity, but the consequence of soluble phosphates lack [137].

5.4.2. Copper

Although the Cu and Ni in nature often occure together and have similar toxic properties, they do not have joint effect on fungi [138]. Copper also forms insoluble sulphids. It's toxicity depends on the affinity for sulfhydryl groups and other tiolate groups that represent reactive centers of many enzymes [99], but also on binding to amino and imino groups [139]. Metallothioneins, γ-glutamyl peptides and other tiolates are involved in the detoxification process in fungi.

5.4.3. Chrome

Cr^{3+} and Al have many common features. By formation of $[Cr(H_2O)_5OH]^{2+}$, chrome lowers the pH of the solution [140]. Cr-sulphids are being hydrolyzed by water, which is why metalothionins and γ-glutamyl peptides can not bind it. Potential mechanisms of detoxification are: 1 - binding to hydroxy-, carboxyl- and methoxy- ligand groups in the form of chelates or organic polymers; or 2 – binding to polyphosphates in the vacuole [99].

5.4.4. Zinc

Zinc belongs to the moderately toxic elements for fungi. According to electronegativity it is between Cu and Al. At low pH it forms acid compounds $[Zn(H_2O)_5OH]^+$ [140]. The most efficient mechanisms of detoxification are binding to γ-glutamyl peptides and polyphosphates of the vacuole, as well as to metallothioneins to a lesser degree [99]. Zn serves as a nutrient in small amounts but is toxic in high doses. Ectomycorrhizal fungi exhibit completely different mechanisms of resistance to Zn and Cu.

5.5. Defence Mechanisms (Resistance/Tolerancy)

Toxicity of any metal depends on adaptability of fungi to environmental factors. Two groups of defence mechanisms are distinguished: resistant/tolerant and sensitive. Some authors recognized two basic defence mechanisms: 1) strategy of exclusion when concentration of heavy metal is on constantly low concentration until the critical one when metal is urgently transported in cell and performs its toxicity, 2) accumulative strategy when metal is actively concentrated in organism [141]. Resistance of fungi towards toxic metals can be defined as the ability of organisms to survive in the presence of high concentrations of metals by applying different extracellular mechanisms of defence as a direct answer to the damaging impact of metals. The defence is usually based on immobilisation of heavy metals using extracellular and intracellular chelating compounds e.g. synthesis of metallothioneins (copper or silver binding in *Agaricus bisporus* [142] or γ-glutamil peptides, mycophosphatin and phosphoserines (cadmium binding in *A. macrosporus* [143]. On the contrary, tolerance is a passive mechanism towards toxic metals that can be defined as the ability of organism to survive in a presence of toxic metals by applying intracellular abilities such as insolubility of cell-wall, production of extracellular polysaccharides and metabolic excretes which role is to detoxify metals using linkage or precipitation [144].

Moreover, existing tolerance/resistance on specific metals thus not include the analogues ability towards the other metals, implying these properties of fungi as strongly metalospecific. If multiple tolerance exists, then it reflects the toxic level of many specific metals in substrates ([145], [146]).

Biological mechanisms that are involved in these processes are: 1) extracellular precipitation, 2) formation of complexes and crystallization, 3) transformation of metals by oxidation, reduction, mutilation and dealkylation, 4) biosorption in cell wall, on pigments or extracellular polysaccharides, 5) in capacity for transport or completely membrane insolubility for specific element, 6) active transport of metals from the cell and 7) intracellular compartmentation and precipitation. Different organisms use directly or indirectly many different defence strategies against toxicities. Many fungal species among all taxonomic groups can be found in heavy metal polluted areas and soils contaminated with Cu, Cd, Pb, As, Zn. This pollution has impact on fungi by reducing the diversity and density of fungal populations or by strong selective pressure affecting the population of resistant/tolerant speceis [99]. Some species become tolerant to heavy metals, prevealing in such areas. The species *Laccaria laccata* and *Schizophyllum commune* are most often found in areas with high metal contents (([55] [134], [147]). In unpolluted areas, examples of resistant strains indicate that survival of these organisms is more dependent on intracellular fungal features then on adaptations on physico-chemical environmental factors. Humus or lignin degrading fungi in polluted areas are able to select ecotypes with tolerance to Co, Cu, Hg, Ni, Zn if produce tirosinase, whereas polyphenol degrading fungi and terrestrial fungi (e.g. *M. oreades*) develop tolerance towards metals [148].

5.5.1. Extracellular Mechanisms (Precipitation and Complex Forming)

Many extracellular products (e.g. organic acids: lemon acid or oxalic acid) of fungi can form chelate complexes with heavy metals or precipitate it by forming insoluble crystals of oxalate around cell walls or in extra cellular medium. White-rot fungi produce extracellular hyphal sheat, composed mainly of polysaccharides, including β-1-3 with β-1-6-linkages

which binds calcium oxalate crystals. In white-rot and brown-rot fungi, extracellular and cell-wall associated binding is more important [101]. Oxalate is the most typical metal chelator which is mostly produced in brown-rot fungi (e.g. *D. quercina*), but also in some white-rot fungi (e.g. *P. ostreatus*, *P. chrysosporium* and *T. versicolor*) [149]. Many filamentous fungi excrete molecules of high affinity towards Fe, called syderofors, in order to complex Fe in a form of helates from the extracellular matrix ([150], [151]).

Cell wall is the first barrier of fungi towards heavy metals since it represents a place of controlling uptake of both soluble substances and water in the cell. Physico-chemical interactions comprise the following processes: ion exchange, adsorption, complex formation, precipitation and crystallization [152]. A first step in the interaction between heavy metals and a cell is a connection to the groups that contain oxygen (carboxylic, phosphate, phenolic, alcoholic, hydroxyl, carbonyl and metoxyl). Chitin and chitosan are the most important polysaccharide constituents of the cell wall and the major donors of functional –NH groups that are dedicated to the fixing metals Cu^{2+}, Co^{2+}, Cd^{2+}, Mn^{2+}, Zn^{2+}, Mg^{2+}, Ni^{2+}, Ca^{2+} ([153], [100]). Another group of heavy metal binding compounds produced by fungi are melanin and phenol polymers associated with the cell wall which have also ability to compex metal ions that decrease in the following order Cu>Ca>Mg>Zn ([154], [155]). Some fungal melanins are efficient bioabsorbers of copper. Melanin is a pigment that is important in the cell answer to the stress conditions and is located in the cell wall or on the extracellular surface in a granular form.

5.5.2. Intracellular Mechanisms

Intracellular mechanism of tolerance comprise processes of detoxification based on the: 1) chelation of metals in the cytosol with organic acids (malonic and citric), 2) forming salts between inorganic acids and heavy metals, and 3) accumulation of heavy metals in the cell organelles, especially in vacuole.

Metalollothioneins are citoplasmatic proteins, 10kD, which synthesis is induced by high concentrations of metals Zn, Cd, Cu, Hg, and Ag [156]. They are small polypeptides wealth in cysteine being able to chelate metals by sulphydril group SH-. Short γ-glutamil peptides are synthesized in cytoplasm by enzyme phytochelatin-synthase, containing three amino acids - glutamine (Glu), cystine (Cys) and glycine (Gly), and further transported to vacuole [156]. They are known as phytochelatines and represent the main detoxifying elements in filamentous fungi [157].

In white-rot fungi neider phytochelatins nor metallothioneins are registred, but they might be replaced with onother types of peptide or protein molecules found in certain species (e.g copper and cadmium binding peptide was found in *P. ostreatus* or copper binding peptide from *Grifola frondosa* with MW of 2240 Da ([101], [132]).

Fungal vacuoles have the most important role in macromolecules degradation, storage of metabolites and ions of cytoplasm and in regulation of pH homeostasis since they are capable of regulation of both essential and nonessential metals concentration in cytosole. Polyphosphates in vacuole are dedicated for binding two-valent cations Mg^{2+} and Mn^{2+} [158] by localization (compartmentation) of these elements Ca^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}, Co^{2+}, Fe^{2+} inside the cell.

Fungi are capable for chemical transformation of metals and metalloids by the processes of oxidation, reduction, metilation and dealcilation, thanks to the enzymes they produce, making them less toxic or vaporizing them from the environment ([159]). Moreover, fungi are

able to change local microhabitats causing changings necessary for efficient degradation of pH or metabolic excretion. *Phaeolus schweinitzii* can degrade trimethyl lead [160].

Heavy metals, in general, are potent inhibitors of enzymatic reactions, hence in white-rot fungi these metals influence extracellular enzymes involved in wood degrading process. Low concentrations of essential heavy metals are necessary for the development of these enzymes since they are a constitutional part of its structure (e.g. Mn is incorporated in a structure of mangan peroxidase - MnP, Cu is a cofactor of the enzyme laccase). The positive effect of Cu addition on the production of laccase was observed in various fungi but was proven to be strongly regulated at the level of transcription in *Trametes versicolor* [161].

5.6. Accumulation of Metals in Macrofungi

Although macrofungi have been traditionally used in human nutrition for centuries, in the last decades fungi were pronounced to be especially good sources of healthy food [162], natural antioxidants ([119], [163], [164]) or other biologically active compounds, including antimicrobials, cytotoxic and immunomodulative substances ([118], [165]). Hence, its consumption is lately increasing in many countries. Since fungal species could be an important portion of the human diet in the future, it is necessary to investigate more the chemical constituent and nutritional quality of both wild and cultivated mushrooms.

Screening of occurrence of trace elements in fungal sporocarps has been carried out due to the two main reasons: they can be used as bioindicators of environmental pollution, especially soil contamination [166], and some edible or medicinal species can accumulate high levels of heavy metals making its consumptions as detrimental to human's health [167]. Furthermore, the knowledge on heavy metal levels in mushrooms is alo very important for assessing their transfer along the food chains.

Some authors discuss macrofungi only as rough bioindicators of soil pollution with heavy metals [168], because of their tremendous bioaccumulative ability. This makes them unapropriate for usage for an exact assessment of soil pollution at the given place; but more represent an useful tool for distinguishing polluted versus unpolluted areas. By comparing the two Slovene areas (surroundings of thermal power and lead smelter) with high emissions of heavy metals with respect to levels of As, Cd, Pb and Hg in 16 fungal species, it was found that the average heavy metal levels (mg/kg d.w.) coincides fairly well with data on heavy metal emissions as well as their burdens in soil and other biota (e.g. vegetables, domestic animals, roe deer), confirming macrofungi as a good biomonitoring tool. Although physiological mechanisms of uptake and accumulation of heavy metals and radionuclides in fruiting bodies of macrofungi are rather speculative, the fact that macrofungi can be used in human nutrition makes this problem very important for the future.

Excessive levels of heavy metals (Pb, Zn, Cd, Hg) in sporocarps of macrofungi, mostly Basidiomycota, can be frequently noticed in polluted urban or industrial environment but also in forest ecosystems that are polluted ([169], [170]). Mostly investigated macrofungi are ectomycorhizal species [171], that are in tight connection with wood, thus making a great impact on surviving and productivity of forests in polluted areas influenced by acid rain, chemicals and minerals that are applying in agriculture. On the contrary, lignicolous fungi were far less analyzed due to the fact that their dominant role in forest ecosystems is in maintaining balance since they are wood parasites holding attention of phytopathologists.

During the last decade of 20th century, important changes in populations of Basidiomycota fungi was noticed, mostly a decrease and loss of many species in central Europe [171], and in south Scandinavia [169]. By comparing polluted and unpolluted areas it is concluded that the main cause of this situation is a devastating consequences of air pollution. It influences dramatically changes of forest soil, such as acidification, decline in pH, exhaustion of buffer capacity, and increased mobilization of some ions (eg. Al becomes soluble), thus increasing their concentration in soil solution and making them available for the fungi [148]. These changes inactivate normal metabolism of mycorrhizal species, leading to inabilities of making positive symbiosis with secondary roots of wood leading to lethal consequences [171].

High concentrations and bioaccumulation of different major and trace elements were reported in the European forests ([172], [99], [173], [128], [174], [175], [176]) and also in Japanese forests ([177], [178]) and in Turkey [179]. Many of them demonstrated that mushrooms tended to accumulate Cu, Zn, Rb, Cd and Cs. Kalac *et al*., [128], observed that Hg, Pb, and Cu were accumulated by tericolous *Lepista nuda* and *Lepiota rhacodes*. Higher concentrations of Pb, Cd, Zn and Hg are found in macrofungi (prevalently ectomycorhizal that are in close contact with roots of wood) from urban or industrial areas ([169], [170], [171]) but also in wood ecosystems that are influenced by contaminators. Data on the general patterns of macro- and microelement accumulations in fruiting bodies of wild-growing lignicolous fungi are scarce ([55], [175]).

According to data based on metal content profiles of 92 mushrooms species [132], different mechanisms are involved in uptake of heavy metals by fungi. It is assumed that uptake ability is genetically coded at a genus- or species- levels beside the physicochemical influence only if the standard procedures are established, e.g. evaluation of contamination, substrate pH and mushroom growth. Saprotrophic terrestrial species, especially the genus *Agaricus* exhibits a strong affinity to Cu, Ag and Cd. Also wood-decomposing species, including mycorrhizal, or those frequently growing in forest area (Boletales, Aphylophoralles, Auriculariales, Lycoperdales, Sclerodermatales, Tremellales) have a tendency to accumulate Cr, Mn, Se particularly *Boletus*, *Suillus* and *Xerocomus* and Pb [132].

Atmospheric deposition of heavy metals from air (fumes, dust and aerosols), especially in the case of Cd, Pb and Hg, is evident in wood-decaying fungi (mercury in *Pleurotus eryngii*, lead in *Fistulina hepatica*) that possess perennial fruiting bodies of huge size. This indicates the importance of wood decaying fungi as bioindicators of air pollution. On the other hand, there are comprehensions that none of the fungi can be considered as effective bioindicator of heavy metal contamination, although the fruiting bodies may serve in determining level of pollution on field survey ([180] [181] [182]). In a recent study that analyzed concentrations of neodymium, lead, thorium and uranium in wild-growing macrofungi, the species specificity was determined as dominant factor of accumulation [166]. Only thorium and uranium were highly incorporated in wood-decaying fungi *Hypholoma fasciculare*, pointing to the substrate composition as an important factor to be considered as it was indicated by several authors [182]. According to previous study which dealt with macro and microelements concentration in tericolous and lignicolous fungi from 5 locations in National park Frushka Gora in Serbia [176], cluster analysis classified fungi mostly by location. The same species from different locations contained different mineral contents, indicating that the accumulation ability is not only genetically coded but also influenced by environmental factors.

Table 1. Data on mean content of nine trace elements (mg/kg d.m.) in wood-decaying mushrooms from unpolluted areas published from 2000-2011

Species	Ca	Mg	Fe	Zn	Cu	Cr	Pb	Mn	Cd	References	Ref.	Region
P. squamosus			139.00	71.00	23.00	9.50	0.80	19.00	0.85	Sarikurkcu, 2011	167	
F. hepatica		898.3	38.90	34.43	7.38	4.79	0.14	7.19	0.07	Ouzouni, 2009	184	Greece
	1640.0	969.0	41.1	50.2	30.9	2.41	42.7	26.8	2.52	Michelot et al., 1998	132	France
M. gigamteus	792.0	1510.0	267	48.7	21.9	0.9	20.8	7.07	3.62	Michelot et al., 1998	132	France
	440,59	2045,45	2504,36	44,21	9,52	7,75	3,25			Karaman , 2002	223	Serbia
Agrocybe aegerita							8.2		27.8	Cocchi et al., 2006	227	Italy
Agrocybe praecox	1680	1250	179	66.6	19.8	3.67	32.4	65.9	3.05	Michelot et al., 1998	132	France
Armillaria mellea							11.5		17.6	Cocchi et al., 2006	227	Italy
			480.9	90.3	30.8		1.0	31.3	0.3	Ita et al., 2006	185	Nigeria
		1063.1	499.0	54.12	17.38	4.20	0.49	55.59	1.67	Ouzouni, 2009	184	Greece
Armillaria tabescens		1150.7	60.40	64.45	17.47	4.37	0.79	11.18	1.80	Ouzouni, 2009	184	Greece
A. polymyces	564,05	1175,70	931,24	52,79	29,51	1,94	3,56			Karaman , 2002	223	Serbia
C. cibarius							11.3		4.2	Cocchi et al., 2006	227	Italy
							4.86			Campos et al., 2009	166	Spain
C. cornucopioides	1940	1000	426	165	49.3	1.94	30.7	223	2.06	Michelot et al., 1998	132	France
		866.3	118.2	54.29	32.49	1.57	nd	22.09	0.38	Ouzouni, 2009	184	Greece
Hirneola auricular-judae							31.3		1.6	Cocchi et al., 2006	227	Italy
	5051	979	58	26.6	1.8	2.3	29.5	23.6	2.1	Ouzouni, 2009	184	Greece
Auricularia mesenterica	5450	980	187	76.5	12.3	3.74	47.4	21.3	3.99	Ouzouni, 2009	184	Greece
Polyporus frondosis			731.6	120.1	34.4		0.4	37.3	0.2	Ita et al., 2006	185	Nigeria
G. applanatum			560.7	137.4	60.8		0.7	25.8	0.3	Ita et al., 2006	185	Nigeria
	4135,5	946,4	446,32	25,14	15,90	4,42	3,53			Karaman , 2002	223	Serbia
G. lucidum			604.8	60.1	43.8		0.7	30.4	0.3	Ita et al., 2006	185	Nigeria
	564,05	1175,7	2290,9	34,87	7,24	3,98	3,72			Karaman , 2002	223	Serbia
Pleurotus sapidus			473.5	98.4	39.2		0.8	28.4	0.1	Ita et al., 2006	185	Nigeria
Pleurotus ostreatus			407.7	90.6	45.9		0.4	39.8	0.3	Ita et al., 2006	185	Nigeria

Table 1. (Continued)

Species	Ca	Mg	Fe	Zn	Cu	Cr	Pb	Mn	Cd	References	Ref.	Region
	625,80	1114,39	148,10	111,07	5,20	3,52	<4,17			Karaman , 2002	223	Serbia
L. sulphureus			337.5	95.1	18.8		1.1	19.5	0.2	Ita et al., 2006	185	Nigeria
	242,07	1020,40	553,01	57,93	9,71	2,74	<6,39			Karaman , 2002	223	Serbia
Nectria innabarina			277.2	30.1	29.3		1.9	19.3	0.2	Ita et al., 2006	185	Nigeria
Polyporus brumalis	2700	677	81.6	72.9	17	3.8	45.2	63.4	3.23	Michelot et al., 1998	132	France
H. fasciculare	2240	728	160	82.4	28.4	3.3	33.6	16	2.7	Michelot et al., 1998	132	France
							3.50			Campos et al., 2009	166	Spain
Xylaria polymorpha	1750	699	55.2	71.8	12.7	1.3	28.4	14.9	1.96	Michelot et al., 1998	132	France
O. olearius							3.60			Campos et al., 2009	166	Spain
	418,86	1544,52	603,82	47,01	24,57	2,22	4,53			Karaman , 2002	223	Serbia

Analysis of microelement content in 22 species was found to be specific and depended dominantly on availability of these elements from their substrates to fungi. It is revealed that specific accumulators (e.g. ecotypes) of particular element were created as a defense mechanisms in the course of the evolution, or as a consequence of stress adaptation by which they could exclude or amortize unfavorable effects of heavy metals presence in the environment [183].

The mycelium network provides extensive contact with substratum and optimum absorption of nutrients. It is designed to accumulate all kind of elements, including heavy metals in its sporocarps and reach much higher concentrations than those of the substrate [166]. In as much as a mycelium has the great surface of hyphae that could absorb and accumulate metals, the majority of them (Cu, Zn, Cd) are captured in fungal biomass in the layer of humus. Basidiomycetous fungi degrade the upper layer of humus which containes polyphenolic compounds (lignin, humic acid, fulvic acid, humin). Enzymes (phenoloxidases) are dedicated to effectiveness of ion binding and chelates forming via ion exchange. With regard to this the high tolerance against heavy metals can be realized among the fungi that have ability of polyphenol degradation [148].

Several studies have pointed out the importance of specific elements to fungal strains. The mean macroelement and microelement concentration across all terrestrial and lignicolous fungi tested by Rudawska and Lewski, [175], was in the following order: N>K>P>S>Ca>Mg>Al>Zn>Fe>Mn>Pb>Cd, or in the similar order in the study of Ouzouni *et al*., [184]: Mg>Fe>Zn>Mn>Cu>Ni>Cr>Co>Pb>Cd. For ten wild edible mushrooms analyzed by Ita *et al*. [185], the heavy metal accumulating potential generally decreased Fe>Zn>Cu>Mn>Pb>Cd (Table 1).

In a recent work of Karaman and Matavulj [183], tericolous and lignicolous wild growing species were analyzed where the highest mean concentration of macroelements (d.w.) was found for N (3.08%) > K (1.83%) > P (0.3 %) > Na (15.09 mg %) > Ca (2226.85 µg/g) > Mg (1384.24 µg/g) > Fe (928.03 µg/g) > Zn (58.14 µg/g) > Cu (17.60 µg/g) > Cr (3.94 µg/g) and Pb (3.88 µg/g). When transfer factors (TF) for the lignicolous fungi are analyzed, some facts should be take into account. First of all the volume of analyzed material of mycelium is just a part of generally present content of biomass in substratum, and also it is present in longer period of time than it is concerned by this analysis. Also the mycelium is in a possibility to accumulate nutritive substances both from dead (saprothrophic) and live (parasitic) material. The content of mineral and trace elements in fruiting bodies, although they are not in direct contact with the soil, can be influenced by the content in soil via mycelial cords and rhizomorphes. This is the possible explanation of higher element concentrations in fungi in relation to the same in soil. Moreover, saprobic lignolitic fungi are not in direct contact with the soil in contrast to mycorhizal fungi

In previous study of Karaman and Matavulj, [183], the highest level of Fe was detected in the lignicolous species *M. giganteus* (2504,36 µg/g) while the highest level of Zn (139,35 µg/g) was found in the species *Schizophyllum commune*. The highest content of Pb and Cr was found in *C. atramentarius*_(9,72 µg/g, 13,36 µg/g, respectively) reaching toxic concentrations. Species which contained more than 1000 µg/g Fe acted as super-accumulators (lignicolous species *M. giganteus*, *G. lucidum* and *Sch. commune* and tericolous species *C. atramentarius*, *F. velutipes* and *P. vernalis*), whereas accumulators of all microelements were *M. giganteus* (exept Zn), *Sch. commune* (exept Pb), and *G. applanatum* (except Fe and Zn). The species that accumulate all of microelements were those posessing large surphace area of

mycelium due to rhizomorphs - *A. polymyces*, and *O. olearius*, and one strictly lignicolous fungus – *S. hirsutum*, which could be pronounced as a super-accumulator species of microelements, especially Pb and Cr [183]. The best accumulator species were tericolous possibly bioindicator species *C. atramentarius* (especially for Fe, Pb and Cr) and *P. vernalis* (especially for Pb and Cu), due to the highest concentration of Pb in the soil of the urban sampling site. The highest Tf =7.5 for Pb indicates that it was not merely the result of the species accumulator ability, but also, the result of specific chemical composition of soil (at pH<7, lower concentration of Mn and Zn influenced on the higher accumulation of Fe). This is in accordance with the data quoting that saprotrophic species could change physico-chemical composition of environment, showing their influence on availability of other elements in substrate and their accumulation in metabolically active hyphe.

According to the significant variations in microelement concentration among the analyzed species, and between the same species from the different sites, especially Fe and Pb content (i.e. *C. versicolor, S. hirsutum*) we assume that genetically different ecotypes of a species can be distinguished according to the ability of metal uptake from substrata. This phenomenon of so called intraspecific antagonism was already reported for macrofungal population of *Coriolus versicolor* [186]. Similiar situation could be expected in populations dispersed on different habitats. This can partially explain the inhomogeneous chemical content of lignicolous fungi derived from different locations ([176], [183]).

5.6.1. Influence of Mycorrhiza on Increasing Tolerance of Plant to Heavy Metals

Mycorrhizal fungi are found associated with most higher plants in which host plant gives the products of photosynthesis to fungi (especially carbon) while the mycorrhizal fungus supplies the host plant with water, less mobile mineral nutrients (phosphorus), improving uptake of NH_4^+, Ca^{2+}, Zn^{2+}, and SO_4^{2-} ions or supplying amino acids and growth substances by excreting or by hyphae degradation. Besides protection from fungal pathogens and soil toxins [99], fungi protect plants from negative effects of heavy metals. This is accomplished by formation of compact mycelia layers around the root, making barrier between the root and the toxic metals (physiologically) to evolve genetically metal tolerant populations. There are suggestions that major evolutionary role of mycorrhizae is to facilitate the acclimation of plants to local soil conditions [187]. Ectomycorrhizal species arrest heavy metals in cell walls or vacuole by lowering metal concentration in soil solution. In addition, complex of hyphae can protect translocation of metals in plants reducing their toxicities. The two species, *Amanita muscaria* and *Paxillus involutus*, were proved to increase tolerance of *Betula spp.* towards Zn [188]. Otherwise, some fungal species could increase an uptake of heavy metals by plants, suggesting that specific mechanisms are still not known enough.

5.7. Interactions between Radionuclides and Fungi

5.7.1. Radionuclide in the Global Environment and Forest Ecosystems

Artificial radionuclides were discharged into the global environment through nuclear weapons tested until 1963, reaching maximum in 1982 ($^{137}Cs \approx 9{,}6x10^{17}$ Bq) [189]. Soon after the Chernobyl accident in 1986, about $3.8x10^{16}$ Bq of ^{137}Cs decay was released into the

environment [189]. After the radioactive deposition originating from atmosphere by precipitations, a high accumulation of radioactive Cs in living organisms was registered, at first in lichens and mosses and later in fungal species [190]. A high transfer factor from soil to fungal sporocarps was recorded after similar events in Japanese forests [191]. The ability of fungi to take up Cs from the substrate (soil and wood) points to these organisms as promising bioindicators of soil pollution. Dighton *et al.,* [192], suggested that much of the absorbed Cs is biologically bound within the fungal tissue and that they have the capacity of holding all the potentially "labile" soil Cs. The main source of radionuclide for lignicolous fungi is the atmosphere, especially for beryllium that is cosmogeny radionuclide ([193], [183]), confirming that these fungi are good bioindicators of air pollution.

Since most fungi occur in forest ecosystems, literature data dealing with wild-growing species may be found in plentiful supply ([191], [194]). Forests are complex ecosystems composed of diverse plant associations, various vegetative strata and multi-layered soil profiles determining radionuclide biochemistry (transfer of radionuclides) characterized by high variability in contaminated areas [194]. It seems that forests are more endangered than the agricultural areas, taking up the major amount of radionuclides via air [195].

Their nutrition via absorption makes them specific bioindicators of radionuclide contamination. Usually, Cs contamination of fruiting bodies is for a degree of magnitude higher than in vascular plants occurring in the same area ([196], [197]). The elementary composition of fruiting bodies distinguish from the same of plants due to high ^{137}Cs, Cs and Rb concentrations and low Ca and Sr concentrations [178].

5.7.2. Radionuclides in Macrofungi

Fungi represent the greatest living biomass and source of enzymes in the forest soil, in organic horizons in particular, where mostly saprotrophic fungi take part in the process of litter decomposition ([198], [199]). Furthermore, fungi take up both nutrients (stable elements) and radionuclides (^{137}Cs or ^{90}St) from the soil aqueous solution, using the same enzymes via specific carrier molecules as well as energy.

Saprotrophic and mycorrhizal Basidiomycota fungi are able to accumulate radioactive ^{137}Cs ([192], [200], [201], [202]) with low transport rate, forming the major pull of radiocesium in the soil [192]. It is quoted that Cs is dominantly present in the upper surface area of soil at a depth of 0-5 cm and deeper than 5cm ([203], [204]), being mostly found in fungal mycelia in amount of 30-50% [178]. There are estimations that soil fungi act as a sink of radiocesium [205]. This statement is a consequence of binding of Cs, together with complexes of organic matter and clay particles, to mineral surface area, resulting in its low concentration in soil solution and low migration withinin soil profiles [206]. According to Nikolova et al., [207], since the production of fruiting bodies was different in different years due to weather conditions, fungal fruiting bodies may accumulate only between 0.01% and 0.1% of the total Cs available in different years. The atmospheric precipitations are associated with the migration of Cs from the soil surface to deeper layers, provoking higher pollution of saprotrophic fungal species with surface mycelia after several years [208], whereas the symbiotic species with deeper mycelia occurring in deeper soil zones (>5 cm) showed higher amounts of radiocesium after an extend period of time ([178], [209]).

There are many dilemmas about the relationship between the contamination rate, pedological characteristics and different nutritional groups of fungi, suggesting symbiotic and lignicolous species as less contaminated than saprotrophic ones [210]. Besides the Cs

distribution in soil zones, another important factor affecting the contamination of macrofungi by radionuclides or heavy metals is mycelia habitat, namely their localization in soil profiles ([178], [208]). Furthermore, a significant fungal redistribution of Cs in the forest soil is needed during the production of fruiting bodies ([211], [212]). Saprotrophic fungi occurring on decomposing material above or within the surface layers of soil are first to be contaminated following deposition. Mycorrhizal mushrooms living in close association with trees may be the most contaminated in the medium or long after deposition while the contamination of parasitic mushrooms relies upon a degree of host tree/plant contamination [194]. Besides, there might be expected an important fungal redistribution of Cs in the forest soil during the production of fruitbodies [198].

It is quoted that about 90% of fungal biomass is concentrated in soil in a form of mycorrhizas, whereas only 10% goes for fruiting bodies e.g. sporocarps ([213], [214]), implicating the occurrence of ^{137}Cs and other mineral nutrients redistribution [207]. It is documented that radioactive Cs can be completely immobilized by fungi in soils and can be further incorporated in nutritional chain by eating fungal fruiting bodies (insects, snails, deer, goats, cattle etc.).

The translocation of nutrients and radionuclides within a mycelium depends on the nutritional type and fungal species [198]. The ectomycorrhizal fungi form complex rhisomorphs which contain large vessel hyphae and radiocesium might be translocated with bulk flow of nutrients or water along the rhizomorphs. Fungi with rhisomorphs thus contain higher concentrations of radiocesium what is evidenced in *Armillaria* species [198].

The concept of transfer factors and concentration ratios are used to quantify the transfer of radionuclides from soil to fungal fruitbodies, usually expressed as the amount of radioactivity per unit weight, dry weight (Bq/kg d.w.) or on a fresh weight basis (Bq/kg f.w.). The commonly accepted concept of 10% of dry matter of mushroom fruitbody mostly used for calculation [189], seems to be discussible. Since activity levels of fungal sporocarps may considerably vary over a small distance as related to various factors [198], other types of transfer factors are suggested (e.g. aggregated transfer factors - Taq), representing the ratio of the activity of fungal sporocarp divided by the total deposition on soil (Bq/m^2) [198].

5.7.3. Health Risk

Mushrooms are of special interest as they represent naturally occurring foods that are collected in forest ecosystems. The Cs transfer factors to mushrooms are widely variable (3-4 degrees of magnitude) due to species characteristics, mycelia depth and nutritional type of mushroom. It is rather speculative in which part of fungi (mycelia network or fruiting body) is higher accumulation of radiocesium, although there are data pointing to the fruiting bodies that act as the final sink while mycelium only mediates its transport and facilitates dilution of the contaminanat [215]. Therefore the fruiting body and in particular its cap is the final place of radiocesium accumulation. This finding is very important as mushroom sporocarps are mostly used in culinary or as the source of bioactive substances. Fortunatelly, the radioactive contamination can be considerably decreased by soaking or cooking of dried or frozen mushroom slices [189].

Accepted statutory limit for foods is 600 Bq/kg of fresh weight i.e. 6kBq/kg of dry matter (d.m.) for mushrooms [189]. According to the European Communities published Council Regulation (CEC, 1987), the maximum permitted level of ^{137}Cs for mushrooms is 12,5

kBq/kg d.m. while 10 kBq/kg d.m.is recomended by International Atomic Energy Agnecy (IAEA, 1994).

5.7.4. Factors affecting the Radioactive Accumulation in Fungi

The concentration of radioactive Cs generally relies upon the forest type [216], soil pH [217], fungal species [192] and mycelium habitat, possibly representing the most relevant factor [178]. On the contrary, the concentration of radionuclides in fungi is determined by a great number of factors among which the amount of radioactive precipitation on the first week of accident, concentration of stable (non radioactive) or analogous element in soil, soil characteristics (mineral composition, pH) and taxonomic and ecological identity of fungus are emphasized [216] (Figure 4). Long-lived product of fission ^{137}Cs is the most frequently investigated radionuclide.

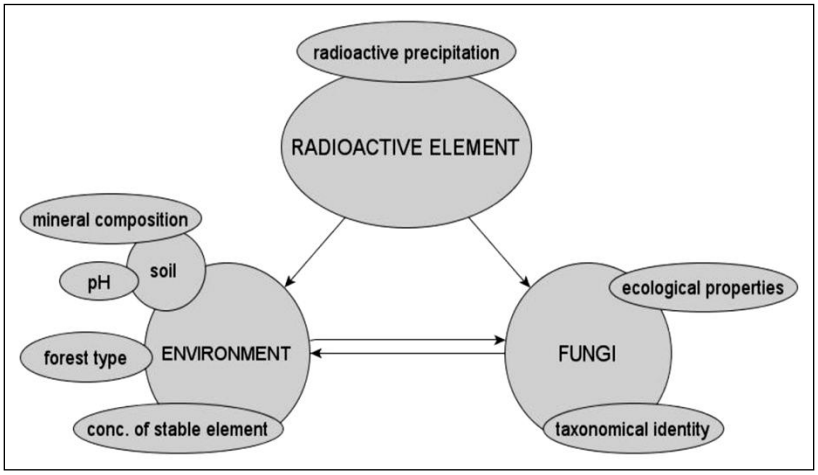

Figure 4. Main factors affecting the radioactive accumulation in fungi.

5.7.4.1. Species Factors (Bioindicators)

There are considerations that the species characteristics (taxonomic and nutritinal) are much more important than the soil characteristics or environmental conditions (e.g. pH, moisture content) ([192], [203], [216]). Some families are known as Cs-accumulators (e.g. Cortinariaceae, Clavariaceae, Entolomataceae and Strophariaceae) while Cs-discriminator are Helvellaceae and Lycoperdaceae [216]. Families, bioindicators of environmental contamination are Amanitaceae, Boletaceae and Russulaceae [210].

After the Chernobyl accident, heavily accumulating species were *Xerocomus badius*, *Xerocomus chrysenteron*, *Suillus variegatus*, *Rozites caperata* and *Hydnum repandum*. In former Yugoslavia, there were only few analyses dealing with the heavy metal concentration in macrofungi of Slovenia [172] and Montenegro [221]. The results presented show that the highest mean values of Cs were found in the species *Cortinarius armillatus* (44 kBq/kg d.w.), *C. traganus* (12,4 kBq/kg d.w), *Rozites caperata* (22 kBq/kg d.w), *Xerocomus badius* (9,3 kBq/kg d.w.), *Laccaria amethystina* (43 kBq/kg d.w.) and in *Boletus edulis* (300 Bq/kg d.w.) while the activity concentration was 110Ag was 500 Bq/kg.

In a recent work of Karaman and Matavulj, [183], the species *P. squarrosa* showed the highest activity concentration level of 55(4) Bq/kg (dry. wt.) and the highest TF values for

essential naturally occurring radionuclide - potassium ^{40}K and the potassium analog - caesium ^{137}Cs, by accumulating the both elements in spite of the fact that the soil was alkaline (pH =6.01) that indicates stronger binding of Cs to soil complexes. Therefore, we imparted special importance to this species as a potential bioindicator species to be used in the radioecological studies of fungi. Since *P. squarrosa* belongs to the family *Strophariaceae* (Cs-accumulator family) ([210], [216]), the presented results confirmed the literature data published elsewhere. However, *P. squrrosa* is the only strictly parasitic species, so the possibility of using nutrients which flow through the host tree xylem is much realistic for real parasitic than for wood-decaying saprotrophes. All these facts recognize parasitic lignicolous species as promising bioindicators. In addition, these data has proved that both the systematic position and the ecological identity (Sap, P, Myc) play an important role in determining the Cs-content of different fungal species.

Furthermore, the activity concentrations of ^{137}Cs in the medicinal species *G. lucidum*, *C. versicolor*, *G. applanatum*, and *M. giganteus* from the Frushka Gora low mountain varied in a narrow range from <2.2 (*C. versicolor)* to 9.4±2.8 *(G. applanatum),* exhibiting values ten times lower than in *P. sqarrosa* (55.4 Bq/kg d.m.) [183]. Accordingly, significant differences within the same species from different localities were not recorded, indicating that the accumulation of radionuclides by fungi is species-specific ([192] [203], [216]). Moreover, the species *G. lucidum* was the only one species that showed high transfer factor for ^{40}K, but low influx of ^{137}Cs, what is possibly genetically determined feature commending this fungi to the attention if used in pharmacology. In addition, a high value of a cosmogeny radionuclide ^{7}Be was recorded in *S. hirsutum* species (140±30) while it was absent in wood bark due to a its fallout on the outside surface of the fruiting bodies. The active surface in this species is maximized because of its pilose upper surface ("Hairy Stereum") ([176], [183]). Various mushroom parts contain various Cs content. High concentrations of ^{137}Cs are recorded in fungal parts with high metabolism rate or abundant cytoplasm. Lamellas showed the highest values of ^{137}Cs, followed by cap and finally the stipe ([202], [219]). It was shown that Cs highly relies upon cap pigments [206].

5.7.4.1.1. ^{137}Cs uptake and Nutritional Fungal Groups

Different taxonomical and nutritional groups of fungi contain different concentrations of radioactive Cs. Great variations in Cs concentration were reported, indicating the following order: mycosymbionts > saprophytic > lignicolous fungi ([210], [216], [220]), although the influx of ^{137}Cs into hyphae of several basidiomycetes showed considerable variations in saprotrophic species exhibiting its highest rates, whereas in mycorrhizal species the lowest ([99], [192]). Lignicolous fungi (e.g. wood-decaying fungi) seem to have the lowest concentration of Cs due to their habitat (wood) showing much lower concentration of nuclides than the soil layers. This indicates that there are two transfer factors, namely the one is a transfer factor operant from soil to sporocarp and the other is a transfer of water and mineral elements through the floem via wood to fungi ([216], [219]). Some authors assume that fungi are accumulators of alkaline metals but not of other fission products derived from Chernobyl [220]. The essential element potassium is chemically very similar to radioactive Cs and therefore can be replaced by nonessential Cs. In addition, some authors think that the accumulation is species specific and that accumulator species do not possess mechanisms

effective enough to distinguish these two elements [202]. There is no correlation between concentration of ^{40}K and ^{137}Cs ([203], [222]).

Influx of Cs in hyphae is very variable (85-275 nmol Cs/g d.w /h) and show higher values in saprotrophic fungi than in mycorrhizal fungi that do not belong to Basidiomycetes [192].

5.7.4.1.2. The Impact of Mycorrhizal Species on Plant Defense against Radionuclides

In mycorrhizal interactions fungi supply plants with carbon and energy, whereas plant supply fungi with water and inorganic substances. In spruce forests, it is noticed that Cs is captured by hyphae sheet and extracellular mycelia, indicating that the potency of ectomycorrhizal species to uptake heavy metals and radionuclides is much higher than that of saprotrophic fungi. Although mechanisms of uptake of K and Cs in cell roots is still not well undestood, it has been documented that mycorrhizoidal spruce seedlings contain lower amounts of Cs than the seedlings lacking fungi mycelia [206].

5.7.4.2. Environmental Factors

Some scientists consider that the Cs uptake is not highly species-dependent, and that it is more affected by location and time of sampling [220]. There are significant variations of ^{137}Cs and ^{134}Cs intake within the same genus as well as among individuals within a species. Since there are great variations of ^{134}Cs and ^{137}Cs intake within the same genus and among strains of the same species [203], some authors state that sites and temporal factor (time of collecting) have the priority [220]. The pH value of soil also have an effect on the availability of Cs, permitting that Cs is more dissolved in acid and more bound in alkaline soils [218]. It is also well documented that the highest availability of Cs to fungi is in positive correlation both with the highest Cs content of soil and the highest humus content (e.g. wither humus zone) as well as the lowest pH values or lower content of essential minerals, especially K. In contrast, lower sand content and highest clay content may cause lower fungal contamination. The highest ^{137}Cs concentration recorded for *G. applanatum* (188±13 Bq/kg d.w.) is explained by specific locality features [176] because maximum Cs values in soil were recorded at the slope margine. This result can be explained by the fixation of radiocesium at the lowest point of terrain profile where the abundance of both the organic matter and humus was reported. Also, the uncultivated forest soil provokes vertical migration of radiocesium, resulting in its binding to the humus layer which makes a large film of organic complexes while leaching to deeper layers is minimized even though it is a very slow process (a few mm per year) [218]. The activity of mycelium in the humus zone is more or less pronounced. Efficient binding of Cs to the clay minerals makes Cs inaccessible for plants, but some fungi can break down and absorb most of the components not available to plants.

6. Antioxidant Enzymes and Free Radical Scavengers as Biomarkers of Environmental Stress in Mushrooms

Biomarkers are defined as a biological response that can be related to exposure to an environmental contaminant. In a broad context they can include measuring such endpoints as

reproduction and growth, or behavioral changes. Concerning aerobic organism defense systems on a cellular level, exposure to pollutants causes the production of potent oxidants and free radicals capable of damaging important cell components such as proteins and DNA. In response, the cell initiates antioxidant enzyme systems and produces free radical scavengers in order to prevent cellular injury and maintain cell homeostasis. The induced biomarker response can then be measured and related to measured concentrations of the contaminant affecting the fruiting body (mycelia) of mushroom.

Concerning our observations dealing with the biological activity (antioxidative, antimicrobial) of lignicolous fungal species collected from northern Serbian Voyvodina Province with respect to the total phenol content or phenolic acid concentration [119], we assumed that the specific bioactivity of fungal species is much dependent on the environmental and stress conditions that are the consequence of processes taking place in the strongly narrow surroundings of the growing sporocarp. These results pointed to the importance of conservation and characterization of fungal culture collection in order to sustain a specific genetical and physiological (metabolic) status that could be further used in mycotechnology or pharmacology.

On the other hand, wood as a substratum shows high potassium, except for *D. tricolor*, *G. lucidum* and, *C. versicolor* where the lowest concentrations even for ^{40}K were found [204]. Our results, ([223], [224], [225]), are in agreement with the results quoted above. In addition to the species registered above, we suggest the following species: *P. gibbosa*, *G. applanatum* and *D. Quercina*, whereas *A. polymyces*, *P. squarrosa*, *O. olearius* and *M. giganteus* should be emphasized as those exhibiting the highest ^{40}K content. The recorded differences might be the result of the morphological and functional differences between these groups of organisms. The last three species presented are mushroom-like fungi with high water content and the highest turnover rate enabling easy transfer of essential and natural radionuclids from soil. In samples of fungal fruiting bodies, ^{40}K concentrations ranged from 45±19 to 1710±120 Bq/kg (dry. wt.) while ^{137}Cs concentrations were between <2.2 and 36±4 Bq/kg. The presented values are in agreement with the literature data on wood-decaying fungi ([204], [226]).

Conclusion

Fungi are ubiquitous in natural environment, especially in forest ecosystems. Achieving processes of lignocellulose decaying and decomposing, wood inhabiting fungi play their vital role in nutrient cycling in the environment, transforming CO_2 and H_2O into the original form.

The occurrence and distribution of major and trace elements both in fruiting bodies of macrofungi (mushrooms) as well as in substrate (soil, wood) samples are inevitable, not only with regard to the basic biological knowledge about elementary composition of fungi in wood ecosystems (physiology and ecology), but, also, from practical aspects of toxicology and environmental protection (conservation of fungal species). Hence these data can be used in order to understand the long term behaviour of radionuclides and toxic elements in forest ecosystems and to speculate on the migration effects of chemical elements, especially the artificial elements in the future.

From the ecological point of view, examination of content of heavy metals and radionuclides in fruiting bodies might indicate the potential application of some macrofungal

species as bioindicators of environmental contamination. Unlike lichens and mosses being mostly bioindicators of air pollution, lignicolous species might be interesting as indicators of substrate (wood) and soil contaminations.

In addition, it is important to ensure that the contamination of wood products is under control, safe for humans. Since the fungal species could be an important portion of the human diet in the future, chemical constituents and nutritional quality of both wild and cultivated mushrooms should be fully investigated. We will emphasize again that the contamination of selected fungal species with heavy metals should be continuously monitored at a local scale where polluted areas are situated as well as at a national scale.

For the potential application of wild growing lignicolous fungi as sources of food or pharmacologically active substances it is recommended to examine the following: 1) species genotype specificities, 2) impact of pollutant content of sporocarp (mycelium) on the environment 3) radioecological analysis of terrain, microhabitats and fruit bodies (mycelia) of fungal species.

Accordingly, potential application of fungi should be ensured by the accurate taxonomical determination of fungal species, analyses of impact of pollutants detected in fruiting bodies (mycelium) of mushrooms upon humans and heavy metal and radio-ecological monitoring of microhabitats. The best and the safest way of revealing all the fungal mysteries is certainly to bring into unity morphological, ecological, biochemical and molecular studies as well as to gather researchers from different fields to work together.

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