

Mycoremediation of Copper: Exploring the Metal Tolerance of Brown Rot Fungi

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In recent decades, fungal roles in bioremediation of toxic contaminants such as potentially toxic elements (PTEs) residing in soil, waste water, and landfills have been studied. Bioremediation is an alternative way to deal with toxic contaminants in the environment. Some decay fungi are able to remove metals by producing metabolites, such as oxalate, which can react with metal ions and generate insoluble forms of metal:crystal complexes. Brown-rot fungi have the ability to produce extracellular oxalate in significant amounts, and this is closely related to chelation of copper by precipitating to copper oxalate crystals. Copper-tolerant brown-rot fungi have a potential role in a bioremediation system by depolymerizing the structure of wood treated with copper-based wood preservatives and adapting to copper through increased oxalate production and formation of copper oxalate crystals. The focus of this review is to suggest that copper-tolerant brown-rot fungi could be a viable option for use in future mycoremediation practices.

Keywords: Copper-tolerance; Mycoremediation; Brown-rot Fungi; Oxalate

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INTRODUCTION

Copper Pollution

Copper pollution impacts a wide range of environmental niches, including soils and water (Lacerda *et al.* 2009; Andrade *et al.* 2010; Kwan *et al.* 2015; Rojas *et al.* 2017). Copper-treated wood waste generally causes pollution in landfills, and it can be harmful to human health, resulting in the potential for cancers and respiratory, pulmonary, and cardiovascular illnesses (Jambeck *et al.* 2006; Mercer and Frostick 2014; Ayala *et al.* 2016). If acute exposure to copper happens, it has the potential to cause death (Hey *et al.* 2009). Copper is used to control plant diseases, especially on grapes (Komárek *et al.* 2010; Andrezza *et al.* 2015). It is documented that copper-based pesticides can affect the health of farm workers by causing neurological, immune, and reproductive diseases (Remor *et al.* 2009). Also, copper mining waste sites are notably problematic due to high copper concentrations (EPA 2017). Potentially toxic elements (PTEs) contaminate certain areas, making it highly available for uptake by plants, water, and soil. It is estimated that approximately 400 million cubic feet of preservative-treated wood is produced annually in the United States (Clausen *et al.* 2014).

Replacement of decayed wood products from homes and buildings costs over \$5 billion annually, with 10% of the annual production of forests going towards replacement of decayed products. Copper-based preservatives are the primary biocide used to protect wood from ground-contact exposure (Freeman and McIntyre 2008). Copper is an effective fungicide against many wood decay fungi, although certain brown-rot fungi have the ability to overcome or tolerate the copper component (Freeman and McIntyre 2008).

Eco-friendly Strategies for Copper Pollution

Using microorganisms to decontaminate soil and water is known as bioremediation and consist of two main methods: contaminated material is treated at the site is referred to as *in situ* and treatment out of the site is called *ex situ* (Rhodes 2013). Removal of contaminated soil can be costly and time consuming. However, if soil or water can be rendered harmless where it lands, the cost of remediation will be reduced and time-manageable. Incineration has the potential to produce dioxins. Extraction of PTEs from soil is also not realistic, as it is unable to completely eliminate copper or other heavy metal from the environment. By contrast, myco- or bioremediation is a viable solution that can provide degradation of toxic organic materials. In addition, mycoremediation has the potential to bind toxic metals and render them inert in the environment. This includes transformation of these contaminants to different non-toxic forms such as CO₂, H₂O, and HCl (Rhodes 2014). PTEs are basically captured by metal-tolerant organisms such as fungi. Certain species of fungi, including copper-tolerant brown-rot fungi, can feed themselves with copper sources, binding copper with a metabolite (oxalate) that it readily produces during decay; such a process can yield inert copper oxalate crystals (Clausen and Green III 2002).

IMPORTANCE OF BIOREMEDIATION

Disposal of copper-based preservative-treated wood waste will be an increasing problem in future years. Development of alternative treatment systems is crucial because of increasing toxicity, leaching, and other potential environmental effects which have a significant impact on disposal of copper-treated wood waste (Lebow *et al.* 2008). Remediation of wood waste is an important step before incineration or landfilling because of public health concerns (Kartal 2003). There are several methods for remediation of treated wood waste such as landfilling, reuse, incineration, chemical extraction, pyrolysis, and mycoremediation.

Mycoremediation is one of the well-known bioremediation techniques that uses fungi to process contaminated water or soil. Fungal mycelium has the ability to secrete extracellular enzymes and break down organic contaminants into carbon dioxide and water. Wood decay fungi play a key role in breaking down toxic components into less harmful forms. Enzymatic activities of these fungi aid in the degradation of complex compounds and can lead to the elimination of toxic materials from the environment. An example of mycoremediation is using mushrooms and macro-fungi like *Pleurotus* species (also called oyster mushrooms). *Pleurotus* species have a high biosorption potential due to their extensive biomass, *i.e.* mycelial production (Vaseem *et al.* 2017). The genus has been reported to accumulate high levels of PTEs and is used for mycoremediation of copper

(Kapahi and Sachdeva 2017; Vaseem *et al.* 2017). Interestingly, some native microfungial strains (*Aspergillus*, *Trichoderma harzianum*, *Clonostachys rosea*) have been isolated from a silver-polluted site indicating silver myco-accumulation from metal contaminated waste-rock dump sites (Cecchi *et al.* 2017). Another example is the assessment of Ni accumulation by fungi for a possible approach to remove metals from soil and water (Cecchi *et al.* 2017).

Removing heavy metals, specifically copper, by using tolerant fungi is an alternative and beneficial option to reprocess wood waste components (Humar *et al.* 2004). Copper-tolerant brown-rot fungi have the ability to overcome the toxicity of copper-based wood preservatives (Fig. 1). Many brown-rot fungi such as *Postia*, *Serpula*, *Fibroporia*, and *Wolfiporia* sp. are known to be copper-tolerant (De Groot and Woodward 1999; Clausen and Green 2003). Copper-tolerant organisms have the ability to immobilize and precipitate copper into copper oxalate, rendering the toxic copper ions inert (Murphy and Levy 1983). The mechanism of copper tolerance has been theorized to involve additional mechanistic pathways, such as trapping metal ions by cell-wall components, shuttling intracellular copper ions *via* transporter pumps to the outside of the fungal cell, managing pH levels during fungal establishment, regulating the acid hydrolysis of cellulose, aiding the reduction of Fe^{3+} to Fe^{2+} in non-enzymatic decay, and facilitating the chelation and detoxification of metal ions (Schilling and Jellison 2005).

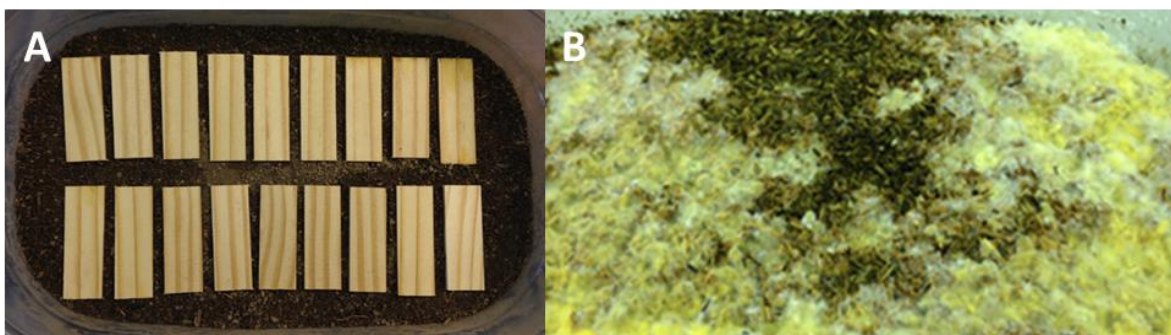


Fig. 1. Decay test set up (AWPA E22-12) with feeder strips (A) covered with copper-treated ground wood exposed to *F. radiculosa* (B)

COMMON COPPER-BASED TREATMENTS

Wood products are used broadly in outdoor applications and residential construction. Wood can be degraded by several different organisms, especially in outdoor applications. Thus, wood products are treated with biocides to prevent degradation. Treated wood is an economical construction material with ecological benefits. Wood preservatives must be effective against a wide range of fungi and termites; be resistant to leaching; be able to penetrate wood and remain in the wood; be resistant to corrosion; repel water; be economically feasible; have low impact on the environment; and have safe handling and use.

Creosote, oil-borne pentachlorophenol (penta), and the water-borne arsenicals, principally chromated copper arsenate (CCA) are called first-generation preservatives (Levi 1973). CCA was the main wood preservative used in residential construction until

the 1990s when some concerns arose about exposure and disposal of arsenic and chromium in the CCA-treated wood (Preston 2000; Freeman *et al.* 2003). In 2004, the Environmental Protection Agency (EPA) limited the use of CCA to industrial applications (Lebow 2004; Lebow *et al.* 2004; Freeman and McIntyre 2008; Lebow 2010). Due to the restriction of CCA, the American Wood Preservers Association (AWPA) continues to regulate a range of chromium and arsenic-free formulations that use copper as the major active ingredient (Lebow 2004; Clausen and Lebow 2011). Copper can inhibit the growth of bacteria, fungi, insects, and mold. Because of these properties, it is currently the main component used to protect wood for residential applications (Freeman and McIntyre 2008). Copper has efficiency against a wide variety of wood-inhabiting organisms including the decay fungi. Copper as the primary form of protection may cause certain problems, such as the development of tolerance or water toxicity. Tolerant organisms have the capacity to grow in the presence of high concentrations of toxic metals. Because of this tolerance, copper formulations are combined with an organic co-biocide to provide greater defenses. Quaternary amines and azoles are the common copper co-biocides (Shupe *et al.* 2008).

Copper is an essential micronutrient and a component of many enzymes, such as laccase. However, in high concentrations it is a potent fungicide. Copper presently is the main biocidal component used in preservative treated wood products, and it is active against soft-rot and white-rot fungi as well as many species of brown-rot fungi; however, certain brown-rot fungi have the ability to overcome or tolerate the copper-component (Freeman and McIntyre 2008). Organic co-biocides, such as quats or azoles, are added to the preservative formulation to inhibit copper-tolerant species. Hastrup *et al.* (2005) defined copper tolerance as 'the capacity of an organism to survive in the presence of high copper ions'. Not all brown rot fungal species are copper-tolerant, and among those that are copper-tolerant, there is variation in tolerance levels, depending on the preservative formulation, fungal species, as well as fungal isolate (Hastrup *et al.* 2005; Freeman and McIntyre 2008). Additionally, the mechanisms fungi use to overcome or tolerate high levels of copper are likely not the same and could vary depending on specific preservative formulations, fungal species, and even fungal isolate. Thus, there is unlikely just a single mechanism employed by fungi for copper tolerance.

Currently, one of the most common wood preservatives on the market for residential construction is ammoniacal copper quaternary (ACQ). ACQ can be found in different formulations (Coudert *et al.* 2013), and these formulations vary with respect to the ratio of copper oxide (CuO) to the co-biocide. ACQ-A contains a 1:1 ratio of CuO to co-biocide, while ACQ-B, ACQ-C, and ACQ-D contain a 2:1 ratio. The co-biocides, called quaternary compounds, or quats, can also vary in different formulations. Didecyldimethyl-ammonium carbonate (DDA-Carb) or didecyldimethyl-ammonium chloride (DDAC) are the biocides found in ACQ-A, ACQ-B, and ACQ-D, and alkylbenzyl-dimethyl-ammonium chloride (ADBAC) is the biocide in ACQ-C (Lebow *et al.* 2004). Another common preservative for the residential market is copper azole (CA), which contains an amine copper and an azole co-biocide. The common current formulation is called CA-B and contains 96% copper and 4% tebuconazole. Organic wood preservatives have been in used in the past as an alternative wood protection choice because of the environmental concerns with toxic metals, which are present in the copper based wood preservatives (Catallo *et al.* 2008). For example, two common azole co-biocides, tebuconazole and propiconazole, are common fungicides and are biodegradable in the soil (Palanti and Susco 2004).

Micronized copper formulations have become alternative preservatives in the current decade. In this system, micronized particles are formed by grinding insoluble copper in water or oil-containing a dispersing agent. In the current market, combinations are called micronized copper quaternary (MCQ) and micronized copper azole (MCA). Micronized copper carbonate formulations usually have a pH 7 to 9, but addition of acids to the compositions may result in a neutral or acidic pH (Freeman and McIntyre 2008).

All treated wood (not limited to just copper-treated wood) has been shown to release small amounts of preservative components into the soil or sediment. These detectable concentrations of preservatives are limited to the areas close to the wood structure. In addition, these leached preservative components typically react with soil components, which limit mobility. Leached preservative levels in the soil can increase over time but haven't had any notable impact on diversity and abundance of the soil flora. There are specific conditions where there is a high potential for preservative leaching, which directly relates to a high potential of metal accumulation, but are mostly restricted to marsh areas with little to no water exchange. The limited water exchange allows the leached preservative metals to accumulate in soil, sediments, and organisms (Lebow 2010).

Simple mycoremediation mechanism represented (Gadd 2004, 2007) by fungal action on organic and inorganic substrates, mediated by enzymes and metabolites, *e.g.* protons (H^+), carbon dioxide (CO_2), and organic acids, and physicochemical changes occurring as a result of metabolism; uptake, metabolism or degradation of organic substrates; uptake, accumulation, sorption, metabolism of inorganic substrates; production of organic metabolites, exopolymers, and biomass; production of inorganic metabolites, secondary minerals and transformed metal(loid)s; and chemical interactions between organic and inorganic substances, *e.g.* complexation and chelation (Fig. 2A)

BROWN-ROT DECAY MECHANISM

Brown-rot decay has often been referred to as non-enzymatic decay because, like some aspects of white-rot decay discussed above, brown-rot decay is first observed as 'decay at a distance'. Brown-rot fungi use both a non-enzymatic and an enzymatic approach to decay wood cell walls, but they lack the main lignin-degrading enzymes found in the white-rot fungi (Kuan and Tien 1993). Thus, as the wood cell walls are being degraded by brown-rot fungi, the wood loses structural integrity due to the breakdown of hemicellulose and cellulose. A modified lignin is left behind, resulting in a dark brown wood that is often cracked into cuboidal sections. Brown-rot decay is the major type of decay found in wood in residential or building applications (Eriksson *et al.* 1990).

Brown-rot fungi enter the wood through the ray cells and axial parenchyma, moving into the cell lumen through pit membranes (Eriksson *et al.* 1990; Daniel 2014). Some species of brown-rot fungi can produce bore holes, but pit membranes appear to be the primary access. Hyphae grow within the cell lumen, producing a slime sheath that binds the hyphae to the S3 layer of the cell wall. Decay begins in the S2 layer, while the S3 layer and middle lamella are not affected in the early stages; thus, decay is occurring away from the hyphae. Characteristics of brown-rot decay include rapid depolymerization of the hemicellulose and cellulose in the S2 layer, resulting in a rapid reduction in wall strength early in the decay process, with only a small detectable loss in weight. There can be

widespread loss of hemicellulose and cellulose when only a small amount of hyphae is present, producing an excess of polysaccharide by-products (Eriksson *et al.* 1990; Arantes and Goodell 2014). All enzymes are too large to penetrate the S3 and S2 layers at the early stage of decay. As decay progresses, the porosity of the cell wall layers increase and breakdown of the other cell layers is also observed. During this process, lignin is modified by demethylation of both the phenolic and non-phenolic portions, and some loss of lignin in the cell walls and middle lamellae is observed (Eriksson *et al.* 1990).

Initial entry of the wood tracheids by brown-rot fungi has been hypothesized to occur *via* chelation of Ca^{2+} ions by oxalate during incipient decay. Chelation of the Ca^{2+} ions allows hydrolysis of the β -1,4 linkages between the polysaccharide units that make up pectin within the bordered pit membranes of wood tracheids (Green *et al.* 1996). Precipitation of calcium oxalate crystals allows fungal organisms entry through the bordered pit membranes (Schwarze 2007). The formation of calcium oxalate crystals also plays a crucial role in regulating the pH of the environment. Management of pH levels and the development of pH gradients between the hyphae and wood cell wall occur as the crystals are solubilized. It is possible that brown-rot fungi regulate pH by utilizing the crystalline and acid forms of oxalate in certain environments (Goodell 2003). During non-enzymatic (incipient) decay when the wood porosity is too small for enzymes, brown-rot fungi produce low molecular mass agents that are responsible for cell wall depolymerization by free radical production ($\cdot\text{OH}$) via the Fenton reaction ($\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \cdot\text{OH} + \text{OH}^-$). Extracellular hydrogen peroxide (H_2O_2) reacts with ferrous iron (Fe^{2+}), creating hydroxyl radicals ($\cdot\text{OH}$) that are able to diffuse through the cell wall and degrade hemicellulose (Xu and Goodell 2001). For the Fenton reaction to occur, brown-rot fungi require certain mechanisms to solubilize iron from iron oxides, to reduce Fe^{3+} to Fe^{2+} , and to produce H_2O_2 (Arantes *et al.* 2009). It has been hypothesized that the production of these Fenton reagents relies on oxalate, which has the capability to reduce Fe^{3+} and promote $\cdot\text{OH}$ production. Schmidt *et al.* (1981) demonstrated that oxalate has the ability to increase the activity of Fenton-based degradation by reducing Fe^{3+} to Fe^{2+} . However, the reactivity of Fe^{3+} is dependent upon the pH and concentration of oxalate in the surrounding areas. Oxalate also has the potential to scavenge iron from iron-oxide complexes by creating a pH gradient between the fungal environment and the cell wall. Once iron is obtained, oxalate forms an iron-oxalate complex, which can diffuse into the cell wall and initiate incipient decay in the S2 layer of the wood cell wall (Arantes *et al.* 2009).

Copper Tolerance

Copper is an essential micronutrient and a component of many enzymes, such as laccase. However, in high concentrations it is a potent fungicide. Copper is the main biocidal component used in preservative treated wood products, and it is active against soft-rot and white-rot fungi as well as many species of brown-rot fungi; however, certain brown-rot fungi have the ability to overcome or tolerate the copper-component (Sutter *et al.* 1983; Freeman and McIntyre 2008). Organic co-biocides, such as quats or azoles, are added to the preservative formulation to inhibit copper-tolerant species. Hastrup *et al.* (2005) defined copper tolerance as 'the capacity of an organism to survive in the presence of high copper ions'. Not all brown rot fungal species are copper-tolerant, and among those that are copper-tolerant, there is variation in tolerance levels depending on the preservative

formulation, fungal species as well as fungal isolate (Hastrup *et al.* 2005; Freeman and McIntyre 2008) (Figs. 3 to 5). Thus there is unlikely a single mechanism employed by fungi for copper tolerance.

Toxic levels of copper can denature proteins (including enzymes) and destroy the integrity of the cell membrane and cell walls (Gadd 1993). Changes that have been documented in some, but not all, fungi when adapting to high levels of copper include: increased production of oxalate, production of a thicker cell wall and an increase in N-acetyl glucosamine (chitin) in the cell wall, and an increase in the volume of the protective mucilaginous sheath (Green and Clausen 2003; Hastrup *et al.* 2006; Vesentini *et al.* 2006; Freeman and McIntyre 2008; Arango *et al.* 2009). The mechanism by which increased levels of oxalic acid confer tolerance is believed to be through the chelation of excess copper ions to the oxalate, forming copper oxalate complexes. These complexes precipitate into copper oxalate crystals on the surface of the wood cell, rendering the copper both insoluble and inert (Woodward and DeGroot 1999; Green and Clausen 2003). The level of oxalate production during copper tolerance depends on preservative formulation as well as species (Arango *et al.* 2009), with some copper tolerant brown rot species showing no increase in oxalate levels. It has been well documented that the copper-tolerant brown rot fungus, *Fibroporia radiculosa*, adapts to copper through increased oxalic acid production and formation of copper oxalate crystals (Tang *et al.* 2013; Ohno *et al.* 2015).

As previously discussed, oxalate is believed to play a very important role in the general mechanism of brown-rot decay by reducing the pH of the fungal sheath, promoting solubilization of the ferric iron, and forming iron-oxalate complexes. However, in the presence of toxic levels of copper, the levels of oxalate produced by the fungus drastically increases. Biosynthesis of oxalate in brown-rot fungi potentially occurs through a coupling of the tricarboxylic acid (TCA) cycle located in the mitochondria and the glyoxylate (GLOX) cycle located in the peroxisome (Dutton and Evans 1996; Munir *et al.* 2001; Hastrup *et al.* 2006). Figure 2A diagrams the inter-relationship between these two metabolic cycles. Oxalate could be produced from excess malate originating from either the TCA or GLOX cycles or from glyoxylate in the GLOX cycle (Munir *et al.* 2001; Tang *et al.* 2013).

Production of Oxalate

The production of oxalate occurs in large quantities by numerous classes of fungi, and is known to be a secondary metabolite of the decay process (Espejo and Agosin 1991; Green *et al.* 1991; Hastrup *et al.* 2005). In CCA treated wood, fungi such as *Coniophora puteana*, *Fomitopsis palustris*, and *Laetiporus sulphureus* have the ability to produce oxalate at different levels, and aid in the bioremediation process (Clausen 2000; Kartal *et al.* 2004a). In addition, the mold fungus *Aspergillus niger* has the ability to remove high levels of Cr, Cu, and As from CCA treated wood, and the secretion of oxalate into culture broth has been observed (Kartal *et al.* 2004b). Furthermore, *Serpula lacrymans*, a brown and dry-rot fungus, is known for its copper tolerance ability (Hastrup *et al.* 2005; Kose and Kartal 2010; De Groot and Woodward 1999).

Brown-rot fungi exposed to wood, an environment with low carbon and nitrogen, have the potential to generate oxalate instead of carbon dioxide from the tricarboxylic acid (TCA) and the glyoxylate (GLOX) cycles (Dutton and Evans 1996; Schilling and Jellison

2005). Oxalate could be produced from excess malate originating from either the TCA or GLOX cycles or from glyoxylate in the GLOX cycle (Munir *et al.* 2001). Isocitrate lyase (ICL) converts isocitrate to glyoxylate and succinate in the GLOX cycle.

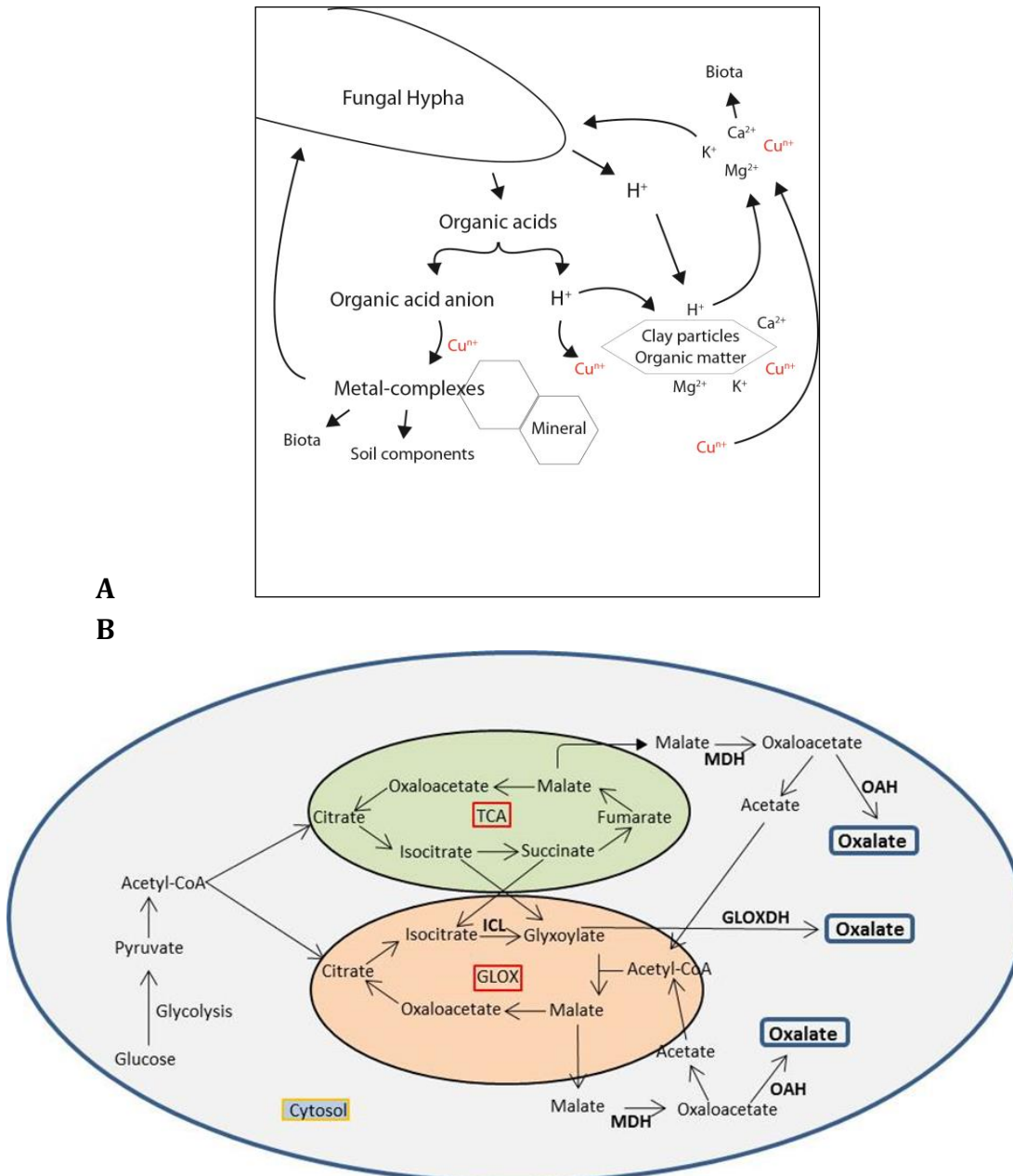


Fig. 2A. Fungal uptake-release mechanism on organic and inorganic substrates (adapted from Gadd 2004, 2007); **2B.** Proposed metabolic mechanism of oxalate production in the brown rot fungus *Fibroporia radiculosa* (adapted from Munir *et al.* 2001 and Tang *et al.* 2013). Tricarboxylic acid (TCA) cycle occurs in the mitochondria (green circle) while the glyoxylate (GLOX) cycle occurs in the glyoxysome (red circle).

If the fungus needs to increase production of ATPs in the TCA cycle or increase glyoxylate levels in the GLOX cycle, both isocitrate and succinate could be shunted between the two cycles by an antiporter protein. Glyoxylate can be converted to oxalate by the enzyme glyoxylate decarboxylase (GLOXDH). Thus, one possible source of increased oxalate is to increase production of glyoxylate and conversion to oxalate (Fig. 2B). Recent genetic studies showed upregulation of both the GLOXDH and ICL genes in *Fibroporia radiculosa* growing on MCQ treated wood (Tang *et al.* 2013). However, ICL was not upregulated on copper citrate (CC) treated wood (Ohno *et al.* 2015) or on untreated wood (Tang *et al.* 2013). High expression levels of GLOXDH are proposed to result in increased oxalate production which forms copper oxalate crystals in *F. radiculosa* (Tang *et al.* 2013). The concentration of oxalate was also found to be high when *F. radiculosa* was growing on CC treated wood. Therefore, oxalate has the potential to accumulate as a function of fungal respiration (Schilling and Jellison 2005).

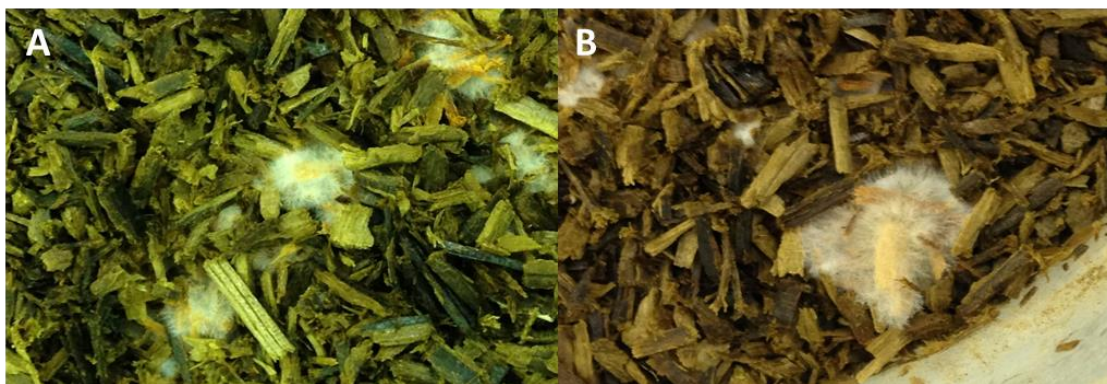


Fig. 3. *F. radiculosa* actively removing copper on copper-treated (A) and copper azole treated (B) ground wood (Akgul 2016).



Fig. 4. *F. radiculosa* actively growing on copper-treated wood for 25 days (Akgul 2016)

Oxalate is believed to play a very important role in the general mechanism of brown-rot decay by reducing the pH of the fungal sheath, promoting solubilization of iron, and forming iron-oxalate complexes. However, in the presence of toxic levels of copper, the levels of oxalate produced by the fungus *F. radiculosa* drastically increases. Figure 3 (A&B) shows that *F. radiculosa* removes copper in a copper and copperazole treatments while it is growing on treated wood. Figure 4 and 5 show that greenish color of copper treated wood changes over time depending on the fungal growth.



Fig. 5. *F. radiculosa* growing on copper-treated wood for 35 days (Akgul 2016)

Genomic Studies on Copper-Tolerant Brown Rot Fungi

In genomic studies of copper-tolerant brown-rot fungi, the mechanism of copper removal is not fully understood. Tang *et al.* (2013) analyzed the genes related to copper-tolerance in *F. radiculosa*. In this analysis, specific proteins with functions related to regulating copper concentrations were characterized, and it was found that three copper-transporting ATPase pumps and one copper homeostasis (CutC) gene were up-regulated during the early stages of decay. The copper-transporting ATPase pumps are largely responsible for preventing intracellular copper concentrations from becoming toxic in non-wood decaying fungi (Weissman *et al.* 2000). Jenkins (2012) measured expression of one of these ATPase pumps in *F. radiculosa* on wood treated with 1.2% ammoniacal copper-citrate over an 8-week period. It was determined that expression of this pump was significantly greater when exposed to the copper treatment at weeks 2 and 4 compared to exposure on untreated controls. They hypothesized that ATPase pump functions to expel toxic copper levels out of the cell during the early stages of decay and serves as a necessary step of initial adaptation to copper (Jenkins 2012). Transcriptome analysis in *Postia placenta*, provided information on the extracellular enzyme system (Martinez *et al.* 2009) and sequencing of the *S. lacrymans* and *Gloeophyllum trabeum*. Such work provided the necessary information for additional gene expression studies (Eastwood *et al.* 2011; Floudas *et al.* 2012). Akgul (2016) showed that the copper treatment have the greatest impact, and regulated 473 genes (compared to untreated) genes in the transcriptomic study of *F. radiculosa*. Also, recently we showed that Aryl alcohol oxidase, catalase, oxalate decarboxylase 2, and copper resistance P-type ATPase pump had higher expression on alkaline copper quat type D treated wood compared with week 1 expressed when the fungus was overcoming the wood preservatives and decaying the wood (Akgul *et al.* 2018). In addition, two genes had high expression at week 5; glycoside hydrolase 5 and glycoside hydrolase 10 when wood strength loss was around 50%. These new studies are important to understand the genes that are involved in the mechanism of copper tolerance and wood decay in *F. radiculosa* (Akgul *et al.* 2018). Further genetic studies need to be done to provide additional knowledge to fungal bioremediation systems. Genetic studies on copper-tolerant brown-rot fungi, such as gene expression analysis of copper removal genes, will improve the understanding of the mechanism of copper removal.

POTENTIAL OF USING COPPER-TOLERANT FUNGI

Disposal of waste copper-based preservative treated wood will be a critical problem in future years. Development of alternative systems become crucial because of increasing toxicity, leaching, and other potential environmental effects which have a significant impact on disposal of copper-based treated wood waste (Lebow *et al.* 2008). Copper-tolerant brown-rot fungi have the ability to overcome the toxicity of copper-based wood preservatives. Therefore, removing the heavy metals by using copper-tolerant fungi is an alternative and beneficial option to reprocess the waste wood components (Humar *et al.* 2004) or any other environmental samples that need to be removed of copper. Future studies need to be done in terms of understanding the effectiveness of copper-tolerant brown rot fungi that have potential usage in mycoremediation.

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