APPLICATION OF EPSILON POLY L-LYSINE

FOR WOOD PROTECTION

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Abstract

Wood is an environmentally friendly material that can absorb carbon dioxide from the atmosphere and is relatively low in acquisition cost compared to other building materials. However, one of the challenges of using wood materials is that it is susceptible to wood-degrading fungi, bacteria and termites, under humid conditions. Epsilon-Poly-L-lysine (EPL) is a secondary metabolite biosynthesized by specific fungi through fermentation. It has been successfully utilized as a natural antimicrobial agent for food preservation. The objective of this study is to test the feasibility of EPL for wood protection. Four common wood-decaying fungi, two wood species, and different treatments were used to test antifungal resistance. Two experiments were conducted in this study: in vitro and in vivo antifungal activity of EPL against wood-decaying fungi. The results of the in vitro antifungal experiment showed that EPL treatment affected fungal hyphae from a smooth structure to a twisted structure and the minimal inhibition concentration of EPL for fungal species ranges from 3 to 5 mg/ml. The results of the wood protection experiment showed that applying 1% of EPL in wood cubes significantly reduced the mass losses in the decay test and also increased the thermal stability of the wood samples. These results indicate a novel approach to wood protection by EPL.

Key words: Epsilon-Poly-L-lysine (EPL), wood protection, secondary metabolite

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Dedication

Last but not least, I want to thank and dedicate this thesis to my family. To my parents and my wife, who have always pushed me to be the best version of myself and to who I owe everything, including this master's degree. I am so lucky to have your support and your unconditional love. Thank you.

Table of Contents

List of Figures

Chapter 1: Introduction

1.1 Objective and motivation

Eco-friendly products as alternatives will reduce the impact of introducing the traditional chemical preservatives in the environment. These products have led to the development of new biological control agents in the field of wood deterioration (Chittenden & Singh, 2009). Traditional chemical preservative is a quick method to fight against the effects of wood-decaying fungi in wood industry. However, the chemical compounds used in preservatives are typically toxic and often affect human health and cause environmental pollution (AWPA Technical Committee P-6, 2017). Several problems with chemical preservatives for wood protection have been identified (Zagury, et al., 2008; Freeman & Mcintyre, 2008). Using bio-based compounds as wood preservatives would benefit not only human health but also our environment.

A promising bio-based compound as a wood preservative is epsilon-poly-l-lysine (EPL). It is naturally secreted by various S*treptomycetaceae* bacteria and some filamentous fungi (Shima & Sakai, 1977). EPL can be considered as an alternative of what based on its specific properties such as high-temperature resistance, high water solubility, nontoxicity, and antimicrobial activity (Shih, et al., 2006). Based on these promising properties of EPL, we hypothesized that EPL can contribute to the treatment efficacy of wood.

The overall goal of this project is to conduct an investigation regarding the feasibility of evaluating EPL as an effective antifungal intervention strategy for wood products, focusing on the efficacy of EPL against wood-decaying fungi and evaluate the durability of EPL on the wood cubes against wood-decaying fungi.

For the efficacy of EPL against wood-decaying fungi experiments: five different concentrations of EPL will be exposed to four different fungal species to determine if the EPL can inhibit common wood-decaying fungi. If so, what is the minimum concentration to inhibit the growth of each fungus? For evaluation of the durability of EPL treated wood cubes against wood-decaying fungi: Wood cubes treated with four different EPL concentrations will be incubated with three different fungi in a conditioning environmental chamber for eight weeks to evaluate the changes of wood mass. The inhibitory effect will be evaluated by less wood mass loss.

1.2 Thesis organization

In this thesis, chapter 2 reviewed wood, wood-decaying fungi, wood preservatives, and EPL application. Chapters 3 and 4 are the major experiments of this project. Chapter 3 is the first experiment to determine the efficacy of EPL against wood-decaying fungi on agar substrates. Chapter 4 is the second experiment to evaluate the durability of EPL-treated wood against common wood-decaying fungi. The conclusion will be presented in chapter 5.

Chapter 2: Literature Review

2.1 Wood

Wood consists of various cell types and chemistries. In general, wood can be divided into hardwoods (deciduous) and softwoods (conifers). In the macroscopic view of wood, there are distinctive areas during the developing process; sapwood and heartwood that are present in both hardwoods and softwoods species. The sapwood and heartwood can be differentiated by the color threshold and served significantly from the other (Domec & Gartner, 2002). Understanding the composition of wood and wood properties inside the wood cells will help to apply wood preservatives in a proper method further and improve wood protection from fungi or insect decomposition.

2.1.1 Chemical composition of wood

2.1.1.1 Cellulose

Cellulose is a linear- chain (Figure 1) polymer that can be found in plant materials, such as leaves, seeds, and wood. Wood consists of approximately 40 to 50% cellulose. Payen, 1838 found that cellulose is the primary polymer of plant cell walls (Payen, 1838; Mondal & Ahmed, 2020). This compound consists of numerous *ß*-D-glucose linked to each other by *ß*-1, 4-glycosidic bond (Sjostrom & Westermark, 1999). The molecular formula of cellulose was determined as $C_6H_{10}O_5$. Typically, the number of glucose units in cellulose molecules is referred to as the degree of polymerization. The cellulose chains in wood have a degree of polymerization (DP) of around 100,000 (O'Sullvian, 1997). Glucose can be treated as nutrient sources for fungi to consume (Costa & Nahas, 2012).

Cellulose consists of fibrils with crystalline and amorphous regions. The chain length of cellulose and hydrogen-bonding pattern are two factors determine crystalline and amorphous (non-crystalline) regions (Williams, et al., 2005; Agarwal, et al., 2021). The crystalline cellulose comprises of well- packed long cellulose chains via hydrogen bonds, whereas the amorphous cellulose has a short and segment length of cellulose chains (Leng et al., 2018). Furthermore, the amorphous cellulose chains are twisted, and the molecules are in a random coil conformation while crystalline cellulose chains are in well-orientation (Fink, et al., 1987). Thus, these are the reasons why they have different behaviors during cellulose hydrolysis.

2.1.1.2 Hemicellulose

Hemicellulose is a kind of cross-linked polysaccharide (Figure 1) that strengthens the cell wall and binds cellulose microfibrils, but cellulose and hemicellulose have distinct compositions and structures. Hemicellulose consists of shorter chains around 500 to 3000 sugar units (B. Esteves, et al., 2013). It composes around 20 to 30 % of the mass of the dry wood but varies by different species and cells. There are five kinds of carbon monosaccharides in hemicellulose: (1) D-glucose, (2) D-mannose, (3) D-galactose, (4) L-arabinose, and (5) L-rhamnose (McKendry, 2002). These monosaccharides can be extracted by water and temperature is important for this process. For example, increasing the water temperature above 180^0C, will dissolve hemicellulose (Lloyd & Wyman, 2003). At temperatures between 200 to 230 °C, 90% of sugars and oligomers from hemicellulose will be recovered (Mok & Antal, 1992). Besides regular sugars, the acidified form sugar, such as galacturonic acid and glucuronic acid can also be found in hemicelluloses (Ebringerová &

Thomas, 2005). They embedded in the cell walls of trees and bound with pectin to cellulose to form a network of cross-linked fibers(B. Esteves et al., 2013).

2.1.1.3 Lignin

Lignin is located in the middle lamella and within the cell wall by strengthening the wood structure (Donaldson, 2001). Lignin accounts for 20 to 33% of the wood mass. The high-molecular-weight of lignin combined with a stable structure leads to its resistance to biochemical and chemical degradation (Sjostrom & Westermark, 1999).

Lignin fills the spaces in the cell wall between cellulose, hemicellulose and pectin components (Donohoe, et al., 2008). The crosslinking of polysaccharides by lignin creates an obstacle for water absorption to the cell wall (Moreira, et al., 2013). Thus, the polysaccharide components of cell walls are hydrophilic whereas lignin is more hydrophobic. Lignin is present in vascular plants. It links to hemicellulose and therefore cross-links different plant polysaccharides, enhancing mechanical strength to the cell wall and helping with the stems' growth so that they can have stronger strength to extend (Chabannes et al., 2001).

The complex structure of lignin makes decomposition slower and should be targeted when developing a new biomaterial. During the wood recovery process, different species of wood materials should be collected separately because the composition and modification of lignin methods are different (Zhang, et al., 2008). Applying hydrothermal degradation is a common method for removing native lignin (Srokol, 2009).

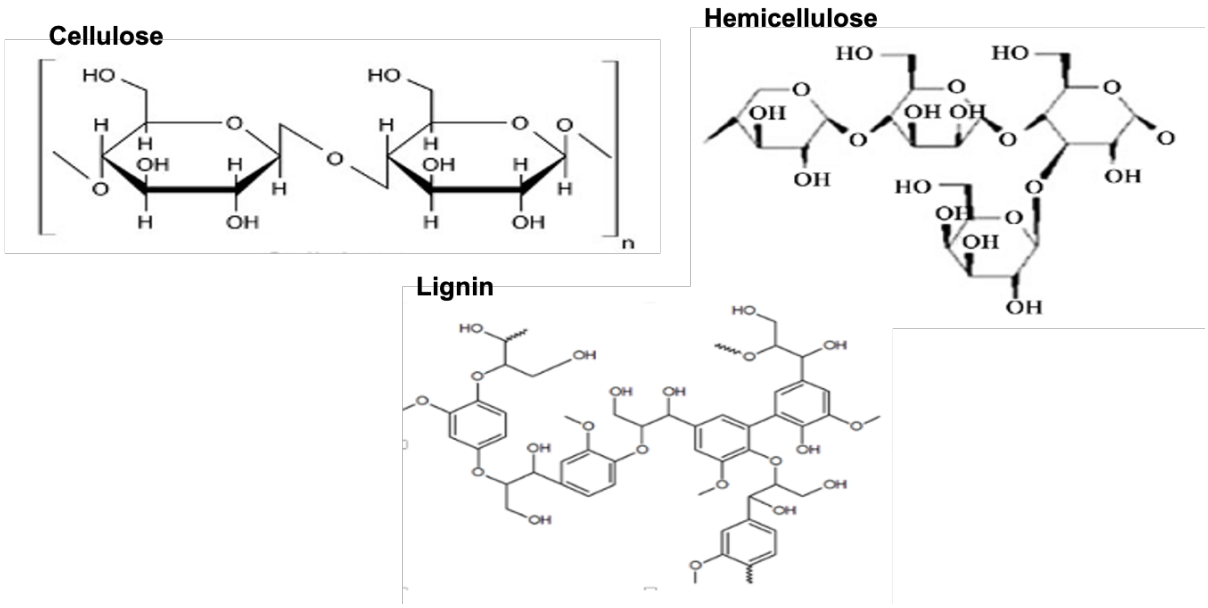

Figure 1. The chemical structure of cellulose, hemicellulose, and lignin (Muktham, et al., 2016).

2.1.1.4 Extractives

Extractives comprise a large number of compounds that are prevalent at the xylem. The conversion of sapwood to heartwood happens only when wood vessels accumulate with secondary compounds, which means the heartwood no longer involves living cells (Maldonado, et. al., 1996 ; Stewart, 1966). The sapwood in living trees is responsible for conducting water, synthesizing biochemicals and storing the carbon in the parenchyma cells to generate needles or leaves. When sapwood gradually transforms to heartwood, the water conducting vessels of heartwood breakdown due to freezing, insects, tension, wind vibration, etc (Yang, 2004). Under these conditions, the adjacent wood parenchyma cells undergo metabolic changes to produce heartwood extractives, such as phenolic compounds and aromatic substances (Yang, 2004). These substances are stored in the heartwood and known as extractives. The extractives are synthesized in parenchyma cells at the boundary of heartwood and sapwood, then infiltrate through pits into adjacent cells (Colin-Belgrand, et. al.,1996) and provide natural protection against decay fungi (Fernández-Costas, et. al., 2017).

2.1.2 Microscopic structure of softwood and hardwood

Wood has a highly diverse microstructure and typically looks like a honeycomb-like structure oriented in the stem direction. The major components of a wood cell for hardwoods are vessels (figure 2) and fibers, whereas the softwoods are tracheids (figure 2). Typically, hardwood vessels are greater than 100 μm in diameter while the tracheid cells of softwood have diameters of 30 to 40 μm (Lichtenegger, et. al., 1999). The water movement inside the wood cells is through tracheids or vessels from one side to another and in transverse direction is controlled by the pit (Choat, et. al., 2006).

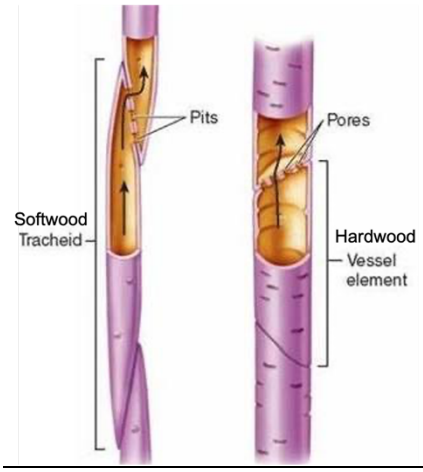

Figure 2. The components of wood cell for tracheid (softwoods) and vessel (hardwoods). The black arrows showed water movement inside the wood cells (Chopite, 2016).

2.1.2.1 Softwood

Softwoods are most commonly known as coniferous trees and they have a relatively simple wood structure comprising axial tracheids and ray parenchyma cells. In softwoods, tracheids occupy a majority of wood structure, accounting for 90% of the wood volume for water transport and mechanical support. From the cross-sectional anatomy, wood cells with rectangular and square shapes are observed. Earlywood typically has thin walls (light color) while latewood (Figure 3) has thicker walls (darker color) (Crivellaro, et al., 2015). Bordered

pits between the tracheids play a role in water flow, which each tracheid overlaps with adjacent cells across both the top and bottom (Yang, et al., 2020).

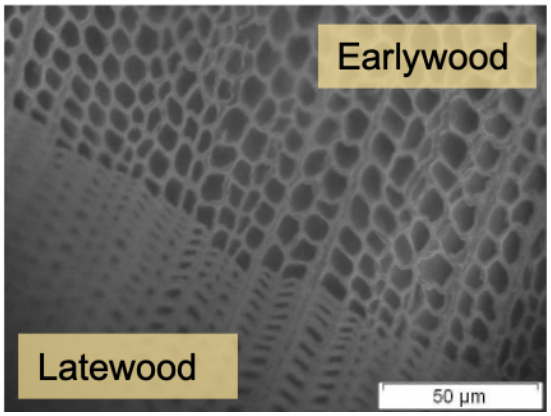

Figure 3. The cross-section of tracheids in softwood (southern pine, *Pinus taeda* L.).

2.1.2.2 Hardwood

Hardwoods have more complicated structure (Figure 4) than softwoods and are composed of vessels and fibers with greater variety of cell shapes and sizes. In hardwood species, vessels are specialized in water-conducting (Mbarga, et al., 2020). The typical range of vessels' diameter is 50 to 200 um and much shorter than the tracheid. It can also form in different patterns where the whole growth ring is scattered with the same size and described as diffuse-porous which means the pores are randomly distributed. In contrast, if the earlywood vessels are much larger than the latewood vessels, then describing as ring-porous (Crivellaro et al., 2015). Fiber is another main component of hardwoods. It is two to ten times longer than vessel elements but shorter than softwood tracheids and about half the width of softwood tracheids (Crivellaro et al., 2015). The role of fiber in hardwood is to provide strength.

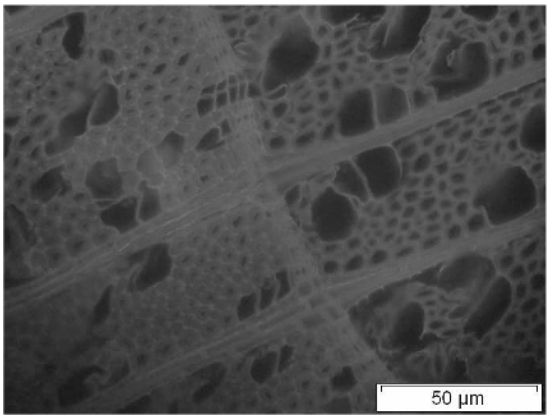

Figure 4. The cross-section of vessels in hardwood (poplar, *Populous tremuloides* Michx.).

2.1.3 Wood properties

Diverse manufacturing procedures need to be considered when producing wood products. Wood density and moisture content are the two most important factors that require monitoring. These two factors will directly affect the shipping costs while calculating cost by weight (Larjavaara, et al., 2016). Besides that, the wood's physical and mechanical properties are decreased by amounts of water absorption and further changes in lumber-drying time, dimensional change, and bond performance (Leblon et al., 2013).

2.1.3.1 Moisture-water relation

The hygroscopic nature of wood means that it can absorb moisture from the air. The moisture content is roughly around 30 to 200% in greenwood (Service & Simpson, 1993). When comparing sapwood and heartwood, the sapwood has typically greater moisture content than heartwood in green softwood. However, the moisture content of green hardwood depends on the species (Altgen, et al., 2016). The relative humidity and temperature are used to determine moisture content inside the wood (Altgen, et al., 2016). The cell lumen and cell wall fully saturated with water refer to maximum moisture content (Popescu & Hill, 2013). The fiber saturation point denotes the point in the drying process that only water bound in the

cell walls and the free water in the cell lumen has been removed from the cell cavities (Almeida & Hernández, 2006).

When wood is saturated with water, the air is replaced by water inside the cell lumen. The water absorption mechanism is called capillary, whereby the air-water creates a curved surface tension inside the cell lumen for water to be taken up (Rowell & Banks, 1985; Khazaei, 2008). During the drying process, to meet an adequate fiber saturated point (around 28 to 30%) is a key for wood to maintain dimensionally stable (Rowell & Banks, 1985). On the other hand, the change in wood dimension happens when moisture below fiber saturated point (Rowell & Banks, 1985). These situations result in the splitting and warping of wood products. Thus, it is crucial to consider the moisture and water relation while exposing wood products in an environment where the moisture content of wood fluctuates a lot.

2.1.3.2 Density

Wood Density is defined as the ratio of mass to volume and is expressed as kilograms per cubic meter (kg /m^{-3}). In wood materials, the volume and mass vary and are dependent on tree species, sapwood or heartwood, age of the tree, and wood moisture content. Therefore, a change in any of these wood properties will alter wood density measurements (Fromm et al., 2001).

2.1.3.3 Bordered pit

The quality of treated wood was determined by how deeply and uniformly of preservatives were penetrated inside the wood cells. Water diffusion through the cell walls and water flow in the cell lumens are the two methods how liquid movement in wood (Watanabe, 1998). Pits are located on transverse and longitudinal sides of the cells which

allow liquid to move across cells and along cells. The components of the bordered pit include torus, margo, and pit aperture (Jansen et al., 2012). The aspiration of the pit aperture by the torus is not only stopping air entry into neighboring tracheids but also affecting the permeability of liquids and wood preservatives when pit aperture closed by a torus (Delzon, et al., 2010; Jansen et al., 2012).

2.2 Wood-decaying fungi

Wood-decaying fungi can decrease the service life of wooden products by secreting enzymes which act to decompose many parts of the wood structure so they can absorb mono-sugars by their hyphae for continuing growth (Isikhuemhen, Mikiashvili, Senwo, & Ohimain, 2014). When fungi digest lumbers or other wood products that built into a house or used as a utility pole will cause a negative economic effect due to the high replacement cost (Rayner & Boddy, 1988). Five factors impact fungal growth: 1) temperature, 2) light, 3) water, 4) nutrient, and 5) oxygen. Controlling these five factors can reduce fungal colonization on wooden products. Therefore, when discussing wood-decaying fungi, it is necessary to understand how these conditions affect fungal growth.

2.2.1 The conditions for fungal growth

2.2.1.1 Temperature

Numerous fungi are responsible for wood decomposition and their optimal growing conditions can vary depending on ecosystem climate regime and vegetation. Typically, fungal metabolism and growth rates are temperature-dependent, which means fungi exist in an extensive range of temperature, but the high peak of performance of growth rate only happens while the temperature reaches the optimal point (Abdel-Hadi & Magan, 2009). The optimal temperature for each fungal species will help them synthesize extracellular enzymes

(M. Rosfarizan & Karim, 2000). If the temperature is lower or higher than the optimal temperature, enzyme activity will be significantly affected (Wills, et al., 2017). Most fungi are mesophilic, which means they are most active at a temperature range from 20 and 35 °C which allows them to expand their fungal hyphae and generate spores (Elmsly & Dixon, 2008). For example, the optimal temperature for *Isaria sp.* growth was ranged from 30 to 40°C (Cabanillas & Jones, 2009) while in another study optimal temperature for *L. curvula* and *H. submerses* growth occurred within a temperature range of 21 to 27°C (Duarte, et al., 2013).

2.2.1.2 Light

Light is a signal factor for reminding the fungal growth to metabolize (Idnurm & Heitman, 2005). In the field, fungi tolerate changing soil conditions. When fungi are exposed to the upper layers in the soil profile, they are closer to a light source and organic horizons of fungi quickly lose moisture. In this case, increased light is an alerting system for the fungi to produce solutes to prevent desiccation (Duran, Cary, & Calvo, 2010). In addition, light can also alter the fungi to diurnal environmental changes (Montenegro-Montero, et al., 2015).

2.2.1.3 Water (moisture)

Fungal growth significantly correlates with the atmospheric moisture in the natural environment (Burdon, 1991).The optimal environment for fungal growth is when the relevant humidity is within the range of 70 to 90 % (Viitanen, 1997). Fungal spores rapidly produce when in a high moisture condition. In contrast, when in an extremely lower moisture condition, the fungal species will lose activity and lead to fungal degradation (Chang, et al.,1995).

2.2.1.4 Nutrient

When the fungi grow, nutrients such as carbon and nitrogen need to be consumed during this process. The decaying fungi use organic materials only when they are easy to digest. Therefore, the cellulose, hemicellulose, lignin, and woody cell walls are becoming the sources for fungi to obtain carbon and nitrogen (Terziev & Nilsson, 1999) (Liverpool, 1987). The mixture of amino acids from nitrogen in malt extract provides a good condition for fungal growth on agar. Fungi uptake the nutrients mainly from the hyphal tips and then diffuse or transport the nutrients inside the fungal mycelium by water (Robson, 1999). The richness of nutrients are critical to support fungal expansion at the early stage of growth. As they grow, the nutrients are depleted over time, and the growth is restricted (Robson, 1999).

2.2.1.5 Air (oxygen)

In the aerobic environment, fungi consume air and oxygen to produce energy, CO_2, and water. When the wood's oxygen content increases, more oxygen is available for decaying fungi (Lindberg, 1992). Therefore, in this living condition, oxygen becomes a beneficial factor for fungal accumulation, which leads to wood degradation. The lowest requirement of oxygen content for wood-decaying fungi to decompose the wood material is approximately 10% *(H. annosum)* to 20% *(S. commune*) (Kreisel, 1961). Thus, spreading water for round timber until the water is saturated in wood cells can effectively reduce the cells' oxygen content and protect wood from the attack of aerobic microorganisms.

2.2.2 Wood-decaying fungus types

2.2.2.1 White-rot fungi

White-rot fungi prefer to dominate the hardwood species and consume the major components of wood, including cellulose, hemicellulose and lignin (Messner et al., 2003). From the macroscopic view, the white-rot fungi can be distinguished by the following features: white-stringy mycelia, white-pockets, or white-mottled wood (Messner et al., 2003). From a microscopic perspective, the white-rot fungi can be classified into either simultaneous or selective white-rot fungi. The difference between these two types of white-rot fungi is that the simultaneous white-rot fungi decompose cellulose, hemicellulose, and lignin at the same time, while the selective white-rot fungi selectively remove or alter lignin (Otjen & Blanchette, 1985). For the selective white-rot fungi, lignin is the priority for decay and this leaves pockets of white. They can remove large quantities of lignin from the cell wall without collapsing the cellulose. One study found that the cellulose were not collapsed while lignin were degraded when observed under polarized light (Anagnost, 1998).

2.2.2.2 Brown-rot fungi

Brown-rot fungi mainly degrade wood polysaccharides, cellulose and hemicelluloses, leaving modified lignin behind (Highley & Murmanis, 1987). In other words, brown-rot fungi have a higher ability to depolymerize cellulose and hemicellulose than lignin. Brown-rot fungi get their name from the brownish color they exhibit after decaying (Highley & Dashek, 2020). Most brown-rot fungi affect softwoods (conifers), particularly in living trees, timbers and wood products in service, whereas white-rot fungi damage hardwoods more frequently (Mali, et al., 2017). After decaying, significant losses in wood strength happen in the early stage of decay and can easily be visualized.

2.3 Wood preservative development history and wood modification review

The use of treated wood as building materials can considerably lower maintenance costs, mitigate climate change, and facilitate sustainable healthy forests (Ritter, et al., 2011). Despite these benefits, wood preservatives may pose a problem due to the loss of chemicals during their service life (Schultz & Nicholas, 2010) and the disposal of wooden products after their serve life (Preston, 2000). The wood preservatives which affect environment and health were reviewed by the United States Environmental Protection Agency (EPA) registration program. In this report, three wood preservatives were identified as high risk of concern associated with the treatment of wood workers, which are creosote, pentachlorophenol (PCP), and chromated copper arsenate (CCA) (Agency et al., 1988).

2.3.1 Wood preservatives

Wood preservatives have traditionally been divided into two general classes: (1) water-borne preservatives, such as arsenicals, chromated copper arsenate (CCA), and (2) oil-born preservatives, such as pentachlorophenol (PCP), and creosote (Schultz & Nicholas, 2010). Creosote is a byproduct composed of a complex chemical while coking coal tar (Schultz & Nicholas, 2010). This preservative accompanies a high risk of creosote leachates, which release into soil and water and move to groundwater when accidental spillage or improper disposal of creosote treated wood occur (Schmitz, 1929). It is primarily used in utility poles, marine pilings, railroad ties, and non-residential applications and accounts for 15% of the wood industry in North America (Schultz & Nicholas, 2010).

PCP is an oil-born preservative that can be easily dissolved in oil. In 1930, PCP was generated in a lower cost of generation processes when compared to creosote, it use to replace the creosote in real wood industrial applications and accounts for 5% in North

America (Schultz & Nicholas, 2010). After PCP, the water-born preservative CCA was introduced during the 20th century. However, in 2003 this preservative was restricted in the U.S. and Canadian wood industries due to the leaching of harmful substances, such as chromium, and arsenic (Zagury, et al., 2008). Several studies determined that the soil near the CCA-treated wood typically has a higher concentration of metal (CrO_3 and CuO) content and hazard index (Guney, et al., 2010; Chirenje, et al., 2003; Stilwell & Gorny, 1997). Additionally, copper-based wood preservatives, such as copper naphthenate and copper-8-quinolinolate, were effective alternatives to PCP due to the leach resistance against decaying fungi and low mammal toxicity during WWII (Freeman, et al., 2006). Since the 1990s, roughly 3% of utility poles and fence posts have been treated with copper-based wood preservatives in the industrial application (Freeman, et al., 2006).

The second-generation, water-born copper-rich systems, such as alkaline copper quat (ACQ), and copper azole (CA) have the same functional performance as CCA, but they have been used more in residential constructions (M. H. Freeman & McIntyre, 2008). However, wood preservatives involved metal hazards (copper) which might affect the corrosion of metal fasteners, aquatic systems, and shorten the service life, thus contaminating entire ecosystems (M. H. Freeman & McIntyre, 2008).

The third-generation systems also known as organic wood preservatives, was developed to enhance the efficacy of the organic chemicals used to protect wood (Schultz & Nicholas, 2008). Developing a new acceptable organic wood preservative has become a major trend to protect not only the wood but also for human health. Several tests need to be evaluated when introducing a new organic biocide to the wood industry. For example, the stability of chemical compounds needs to be tested when exposing it to extreme

environmental conditions, such as high humidity, high radiation, (Freeman, et al., 2006). Secondly, most organic biocides are insoluble in water and might affect the retention variation inside the wood cells (Freeman, et al., 2006). The last consideration is to test the reactions of the combination of other biocides or additives to see if the organic biocides only uses against certain fungi (Wang, et al., 2009). Although developing a new preservative is complicated, the use of organic wood preservatives is a great start to eco-friendly living.

2.3.2 Wood Modification

Wood modification is used to describe a method that application of mechanical, physical, biological and chemical to change the properties of the wood materials, obtaining a better performance of wood dimensional stability, weathering resistance, and decay resistance (Sandberg, et al., 2017). The promising modification of wood methods and widely used in the wood industry are acetylation and furfurylation. The acetylation method involves replacing hydroxyl groups with acetyl groups of acetic anhydride (Dong et al., 2016). The furfurylation wood modification method is realized by the use of furfuryl alcohol (FA) solutions in the catalyst at rising temperature (Lande, et al., 2010). Chemical modification has been applied to modify solid wood properties for specialized applications in the Japanese, European, and North American markets (Johansson, 2017).

The heat-treated wood method was investigated for about a half-century in Europe (Preston, 2003). This method was used to modify the chemical compositions and physical properties of wood. The dimensional stability and decaying fungi resistance of heat-treated wood has been greatly improved (Źivković, et al., 2008). The last method was coating wooden product surfaces with various monomers or polymers to increase utilization of

service lifetime and to prevent damage from microorganisms or natural environmental conditions, such as UV, precipitation, and snow (Rowell, 2006).

2.4 Epsilon Poly-L-lysine (EPL) as a Potential Wood Preservative

EPL is a secondary metabolites generated by isolating *Streptomyces albulus* strain 346 substance from soil (Shima & Sakai, 1977) and has been widely used for a variety of purposes (Zou, et al., 2010). Shima and Shoji reported that the EPL is highly active against a broad large of microorganisms, including fungi, bacteria, and specific viruses (Shoji, et al., 1984). EPL is also been considered a safe food preservative in many countries, such as Japan, Korea, and the United States due to its low impact on living animals (Yasuhiro et al., 2003).

2.4.1 Epsilon Poly-L-lysine

EPL is a high molecular weight compound with 25 to 35 identical l-lysine residues linked by ε-amino and α-carboxyl groups (Shima & Sakai, 1981; Shih, Shen, & Van, 2006). It can resist high temperature, dissolve in water, and has low toxicity to mammals. It also possesses prominent antimicrobial activity against a wide spectrum of microorganisms. The chemical structure of EPL is shown in Figure 5.

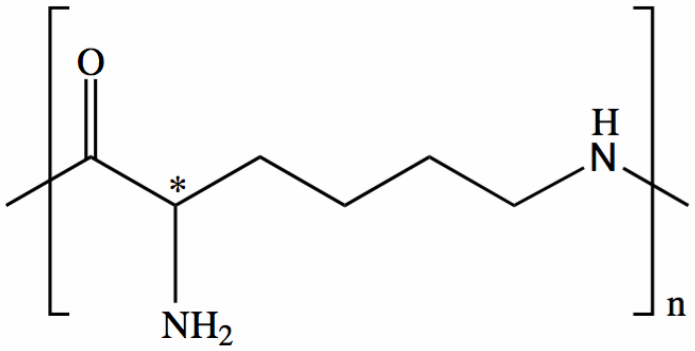

Figure 5. Chemical structure of Epsilon-L-lysine (Amato et al., 2021).

Specifically, the high-temperature resistance of EPL has been demonstrated by its lack of structural change when the solution is autoclaved under 120 °C for 20 minutes or under 100 °C for 30 (Yoshida & Nagasawa, 2003; Shih et al., 2006). The high-water solubility of EPL is another advantage in terms of wood protection. Because water is a low cost solvent and the preservatives carried by water can be easily diffused into wood structures (Thybring, Kymäläinen, & Rautkari, 2018).

For safety and antifungal purposes, EPL has been applied in a moderate concentration to prevent the growth of pathogen in dry meat products (Sofos, 2002) and to avoid infection of disease in crops, such as sprouts and seeds (Sivapalasingam, et. al., 2004). Recently, EPL also used to ease the generation of new hyphae , the synthesis of protein, DNA, and RNA in different fungal species (Rocha, et. al., 2005). For example, the latest research by Rodrigues et al., reported the EPL were used to fight against four phytobacterial species with negatively impacted crop growth. The results showed the plants treated with EPL foliar sprays could effectively suppress plant diseases (Rodrigues et al., 2020).

2.4.2 Antimicrobial method of Epsilon Poly-L-lysine

EPL is a peptide generated by fungi. The peptides also known as antimicrobial peptides (Rodrigues et al., 2020) which collaborated with electrostatic absorption mechanism (Yoshida & Nagasawa, 2003) to inhibit grams, bacteria, and molds. The peptides are found in the immune system of some reptiles, mammals, insects, plants, and fungi to fight against the infection from the outer environment (Blin,et al., 2011). The peptides interact with microbials, causing membrane rupture, and they inhibit intracellular functions, which lead to microbial death (Figure 6).

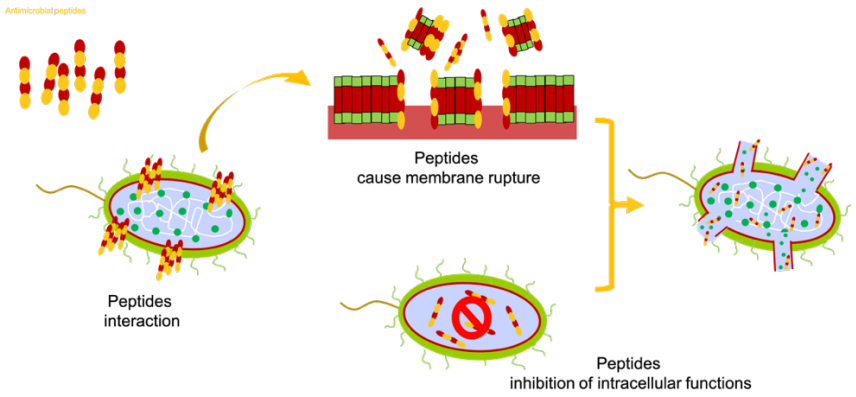

Figure 6. The process of antimicrobial peptides interacts with microbes.

The electrostatic absorption is a mechanism for EPL (peptides) effectively adsorbing the negatively charged surface of microorganisms. The presence of NH_2^+ groups along EPL's backbone provides EPL with a high isoelectric point (pI-9), making EPL has positive charges (Yoshida & Nagasawa, 2003). This positive charge of NH_2^+ groups is a key how this mechanism works. Once the positive charge of NH_2^+ absorbs the microorganisms with negatively charged, the EPL penetrates and distributes inside the cells to create pores, which destroys the membrane. After this, the outer membrane of cells is gradually removed until the cells die (Figure 7). This removed abnormal distribution of cytoplasm is known as fragmentation (Blin,et al., 2011; Shima, et al., 1984).

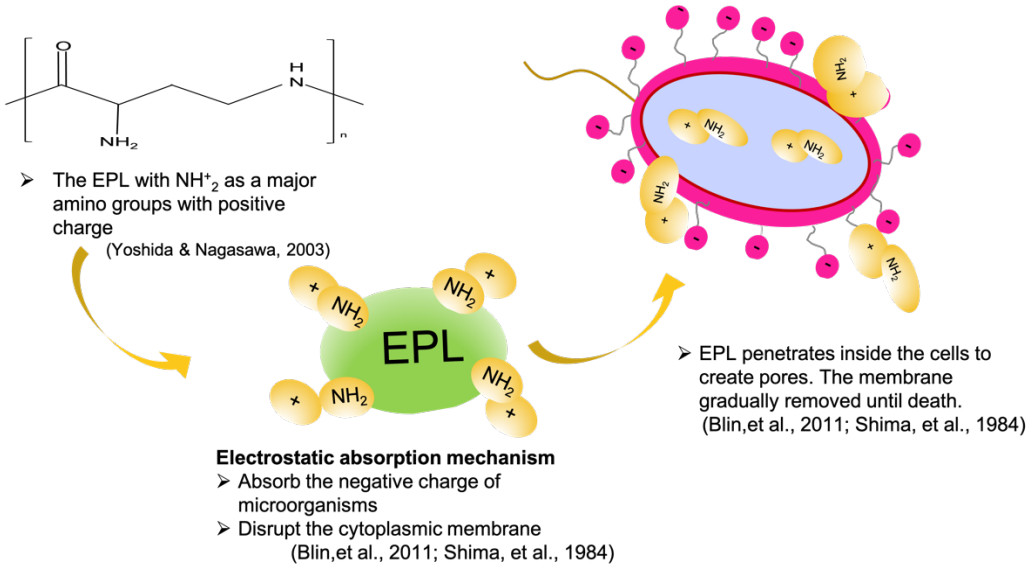

Figure 7. The inhibitions of microbial growth by electrostatic absorption.

2.4.3 Application of Epsilon Poly-L-lysine

In the late 1980s, EPL was approved by the Japanese Ministry of Health Labor and Welfare as a food preservative because of its high antimicrobial properties and low toxicity (Chheda & Vernekar, 2015). For example, the EPL was used to extend the service lifetime in potato salad, noodles, and steamed rice (Yasuhiro et al., 2003). Muto reported that adding 2-3 % of EPL to hamburger patties increased the service lifetime by 20 days (Muto, 2009). In another study by Hiraki (2000), food ingredients can be preserved with EPL at concentration ranging from 1000 to 1500 ppm without cooking them (J Hiraki, 2000).

The antimicrobial properties of EPL can be greatly improved by combining it with other food additives (J Hiraki, 2000). Mixing it with food additives, such as ethanol, organic vinegar, glycine, and emulsifying agents, (Lau, et al., 2006) allows for reduced EPL usage

while maintaining desired protection purposes and the original food taste (Lau, et al., 2006). For example, the service lifetime of raw chicken can be increased threefold longer by submerging a raw chicken in a solution containing EPL of 450 ppm, organic acid of 0.9% and sodium acetate of 1.4% (Muto, 2009).

Research effort has been devoted to the inhibition of food-borne pathogens. In the United States, around 76 million cases of sickness are caused by microbe-contaminated food sources (Mead et al., 1999). EPL can effectively inhibit the growth of the food-borne pathogens *Escherichia coli O157:H7, Salmonella typhimurium,* and *Listeria monocytogenes* (Geornaras & Sofos, 2005). In this study, 200 to 400 ug/ml of EPL, tryptic soy broth, and 0.6% of yeast extract were used to incubate these three pathogens: *Escherichia coli O157:H7, Salmonella typhimurium,* and *Listeria monocytogenes.* Additionally, for the gram-negative pathogen *Escherichia coli O157:H7* and *Salmonella typhimurium,* the 0.025% sodium diacetate or 0.1% acetic acid could enhance the antimicrobial ability (Geornaras & Sofos, 2005). Geornaras and Sofos noted that the combination of additives can be an effective food preservative for controlling food-borne pathogens (Geornaras & Sofos, 2005).

In addition to antimicrobial ability, EPL has been used as dietary fat absorption compound due to its inhibition of pancreatic lipase activity. This means EPL can reduce the absorbing rate of lipids that cause obesity. Another study indicated that when mixing EPL with bentonite, it can prevent periodontal disease (Lion, 2006). Because of the versatility of EPL, there is high interest in expanding the application of EPL to a diverse field, such as agriculture and environmental science. This thesis will test the antifungal efficacy of EPL against wood-decaying fungi.

Chapter 3: In *Vitro* Antifungal Activity of Epsilon Poly L-lysine against Wood-decaying Fungi

Abstract

There has been a growing interest in seeking natural and bio-based preservatives to prevent wood from deterioration during its service life and to prolong carbon storage in buildings. This study aims to assess the antifungal properties of Epsilon Poly L-lysine (EPL), a secondary metabolite from actinomyces, against four common wood-inhabiting fungi, including two brown-rot fungi, *Gloeophyllum trabeum (G.t.)* and *Rhodonia placenta (R.p),* and two white-rot fungi, *Trametes versicolor (T.v.)* and *Irpex lacteus (I.l.).* EPL was amended with malt-agar media at different concentrations of 1, 2, 3, and 4 mg/ml. Our results indicate that these fungi responded differently to the EPL treatments. The minimal inhibitory concentration of EPL against *G.t.*, *T.v.* and *I.l.* was determined to be 3 mg/ml, while that of *R.p.* was 5 mg/ml. EPL treatment also affected hyphal morphology by changing them from a smooth surface with a tubular structure to twisted and deformed shapes. Although only being tested on agar media, EPL has demonstrated high antimicrobial activities against common wood-decaying fungi.

3.1 Introduction

Wood-decaying fungi are important in natural environments and in wood industry. They are unique because of certain fungal species are of special interest in selectively decompose holocellulose or lignin within the cell walls of wood. Wood-inhibiting fungi are the important drivers in the carbon cycle of the earth by decomposing various organic matters into carbon dioxide and water (Li et al., 2019; Treseder, et al., 2016) but also wood decay

processes that cause significant economic losses by facilitating decomposition of living and dead trees as well as wood products (Oliva et al., 2014; Riquelme et al. 2018). Wood-decaying fungi are typically characterized into three types: white-rot (which degrades all components of plant cell walls), brown-rot (leaves lignin intact), and soft-rot (attack higher moisture condition of wood). White and brown-rot fungi are basidiomycetes (Blanchette, 1983) and are commonly found associated with wood decay (Goodell, 2003).

It is important to find approaches to reduce wood decay on structural lumber products that are both economically feasible and environmentally sound. A huge economic loss when buildings have to be replaced due to deterioration in wood strength by wood-decaying fungi. Approaches to protecting wood from various fungal damage include non-fungicidal, fungicidal and biological methods. In particular, the chemicals used in the fungicidal formulations have been challenged by the ever-increasing antimicrobial resistance of the organisms, the rigorous regulations due to the associated environmental pollution and hazards, and the growing awareness of seeking sustainable alternatives (Schmitz, 1929; AWPA Technical Committee P-6, 2017; Buzón-Durán et al., 2019). These issues have led to the exploration of new antimicrobial agents to reduce or eliminate fungal degradation of wood products.

EPL is a secondary metabolite biosynthesized by *Streptomyces albulus* through industrial fermentation (Bankar & Singhal, 2013). It is a linear homopolymer consisting of 25 to 35 L-lysine units, one of the essential amino acids, and is linked by peptide bonds (ε-amino bond) formed by an α-carboxylic acid group of L-lysine and an ε-amino group of another L-lysine (Shima & Sakai, 1981; Shih et al., 2006). As a naturally produced polycationic peptide, EPL has excellent antimicrobial properties against a broad spectrum of

microorganisms, including bacteria, yeast, molds and fungi, under a wide pH range of 2 to 9 (Jun Hiraki, 1999). The biocidal activities are not affected due to high temperature and could last for 20 min at 120^{o}C. EPL is also readily soluble in water, editable and biodegradable (Yoshida & Nagasawa, 2003). It is recognized as safe (GRAS Notice No. GRN 000135) by the Food and Drug Administration (FDA) in 2003. Based on its strong antimicrobial properties and low toxicity, EPL has been extensively used in food preserving and biomedical industries (Hyde et al., 2019). However, the antifungal activity of EPL against wood-rotting fungi has not been reported.

The objective of this study was to evaluate the antifungal activity of EPL against four wood decaying fungi, including two brown-rot fungi, *G.t.* and *R.p.,* and two white-rot fungi, *T.v.* and *I.l.* using malt-agar as substrate. The effect of EPL on the fungal growth rates was monitored for 2-weeks. In addition, changes in fungal morphology are presented.

3.2 Methods and Materials

3.2.1 Materials

Epsilon Poly L-lysine (EPL, >95%, MW is between 3600 and 4300) was purchased from MarkNature (Qingdao, China). Agar, yeast extract powder, malt extract, and potassium hydroxide were obtained from Fisher Scientific.

Two brown-rot fungi, *Gloeophyllum trabeum (G.t.)* and *Rhodonia placenta (R.p)* and two white-rot fungi, *Trametes versicolor (T.v.)* and *Irpex lacteus (I.l.),* were donated by Dr. Juliet D. Tang at the USDA Forest Service Forest Products Laboratory, Starkville, MS and were used throughout the study.

DAPI (4', 6-diamidino-2-phenylindole dihydrochloride), Calcofluor White M2R (1 g/L) and potassium hydroxide were purchased from Millipore Sigma. The calcofluor white was used directly without diluting.

3.2.2 Antifungal assay of EPL-amended malt-agar substrate

The antifungal activity of EPL against the wood-decaying fungi was assessed using malt-agar as a substrate. Briefly, the malt-agar solution was formulated consisting of malt extract, agar and yeast extract at a concentration of 2, 1.5 and 0.2%, respectively, using DI water as a solvent, followed by autoclaving at 120^{o}C for 20 minutes. Then part of the autoclaved malt-agar solution was fully mixed with EPL powder to obtain EPL amended malt-agar solutions of four different concentrations at 1, 2, 3 and 4 mg/ml in a biosafety hood. These concentrations were selected based on a preliminary study (unpublished) that evaluated appropriate EPL concentrations to effectively reduce fungal activity. Both the unamended and EPL amended solutions were further transferred to the Petri dishes (diameter of 90 mm) using sterilized syringes, of which 12.5 ml were cast per plate. After the malt-agar solution solidified, an active growing fungus plug with an area of 100 mm^2 was placed in the middle of each Petri dish. Then the Petri dishes were sealed with parafilm and kept in a controlled chamber in the dark at a temperature of 25^{o}C and relative humidity of 75%.

Fungal growth on the agar substrate was monitored daily for 14 days; as it takes different fungi, about 7 to 14 days to fully cover the whole Petri dish, depending on the fungal strains (Barbero-López, et al., 2020). Photos of each Petri dish from each EPL treatment, fungal species, and replicates were taken every day using a customized box to minimize the stray light in the photos and to obtain clear pictures (Figure 8). The area covered by a fungus was measured using Image J software (National Institutes of Health, Bethesda, Maryland). All the

treatments are in five replicates. A total number of 100 Petri dishes were prepared (5 replicates per treatment x 5 treatments (4 EPL treatments and one control) x 4 fungi (*G.t.*, *R.p.*, *T.v.* and *I.l.*)). The daily fungal growth rate was calculated using the flowing equation:

$$\text{Daily fungal growth rate (\%)} = \frac{A_{day\#} - A_{plug}}{A_{max}} \times 100\%$$

Where $A_{day\#}$, represents the area of the fungus covered on the Petri dish on each day, A_{flug} *means* the area of the plug used to inoculate the plate and A_{max} denotes the theoretical maximum area that could be covered by the fungus. In this study, the diameter of the plate is 90 mm and the maximum area is 6358.5 mm^2.

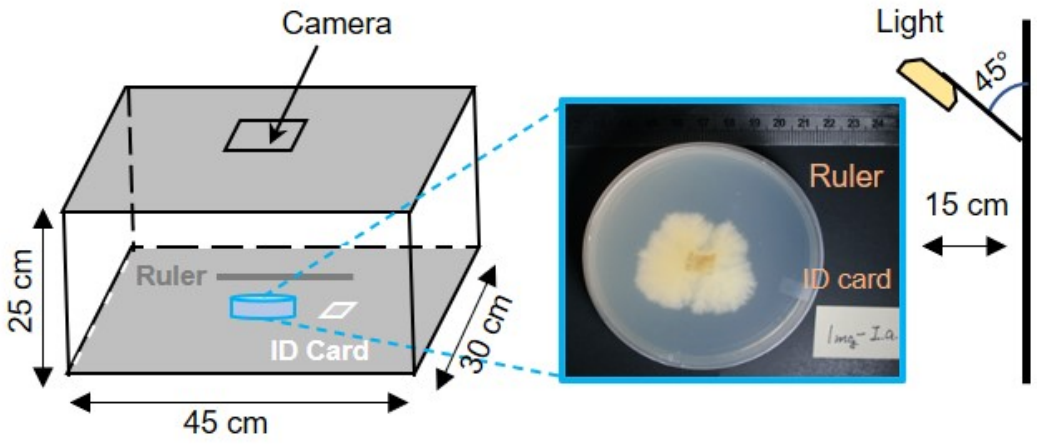

Figure 8. Schematic drawing of the customized box (left), the layout of a Petri dish, ruler and sample ID card inside the box (middle) and light source (right) for photo taking.

3.2.3 Microscopic observation of fungal growth in EPL-amended agar Substrates

The morphological changes due to the effect of EPL were observed using an Olympus microscope (BX51, Tokyo, Japan) under the magnification of 600X. Nuclei observation was evaluated under the blue light excitation. The fungal slide culture was prepared as below (Figure 9). The microscope slides, cover slides and filter papers were autoclaved at 120ºC for 20 minutes before further use. Briefly, a filter paper was placed in a

Petri dish (bottom) and wetted with 2 ml of sterilized DI water. Then two spacers were arranged between the filter paper and a microscope slide to create airflow, followed by placing a 5 mm^2 of solidified EPL-amended malt agar square in the middle of the slide. The EPL-amended malt-agar substrate includes 1 mg/ml for *G.t.*, 3 mg/ml for *R.p.*, and 2 mg/ml for both *T.v.* and *I.l.*. while the control malt-agar substrate was prepared without adding EPL, as previously stated. The four corners of the malt agar plug were then inoculated with the active growing fungal tips (without control malt-agar substrate) and were further adhered to a covering slide. The complete Petri dish set covered by a top plate was then sealed with parafilm and incubated at a temperature of 25 ^oC and relative humidity of 75% in an environmental chamber in dark for 2 days. The cover slides attaching with the newly grown fungal hyphae were then laid on another plain microscope slide and fixed by a drop of DI water. For fluorescence microscopic observation, the solutions, 10 µg/ml of DAPI, 5 µg/ml of calcofluor white and 10% of potassium hydroxide, were used to fix and stain the mycelia. The excess water and solutions were removed using Kimber paper.

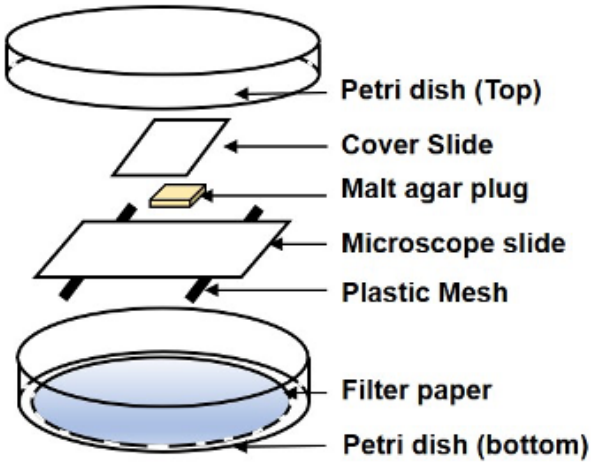

Figure 9. The expanded view of the fungal slide culture.

3.3 Results and Discussion

3.3.1 Wood-decaying fungi growth in EPL-amended agar substrate

Figure 10 shows the effects of different EPL concentrations on the average fungal growth rates of two brown-rot fungi, *G.t.* and *R.p.*, and two white-rot fungi, *T.v.* and *I.l.*, in the malt-agar substrate over a 14-day incubation period and their macro-view was shown in Figure 4. For control, it takes *G.t.* and *R.p.* about 14 and 12 days, respectively, to fully cover the malt-agar substrate. In comparison, the mycelia of the two white-rot fungi, *T.v.* and *I.l.,* completely colonize the agar control substrates on day 6 and 5, respectively, indicating that these two white-rot fungi are more aggressive growers than the brown-rot fungi based on current lab conditions.

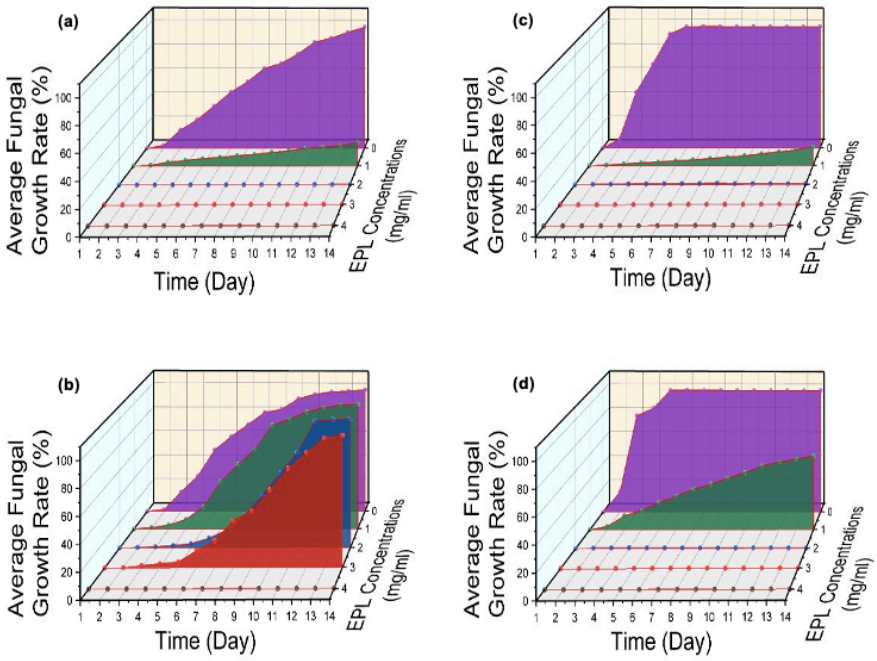

Figure 10. The average fungal growth rates of two brown rot fungi: (a) *G.t.* and (b) *R.p.,* and two white rot fungi (c) *T.v.*, and (d) *I.l,* against different concentrations of EPL in malt-agar substrates over a 14-day incubation period.

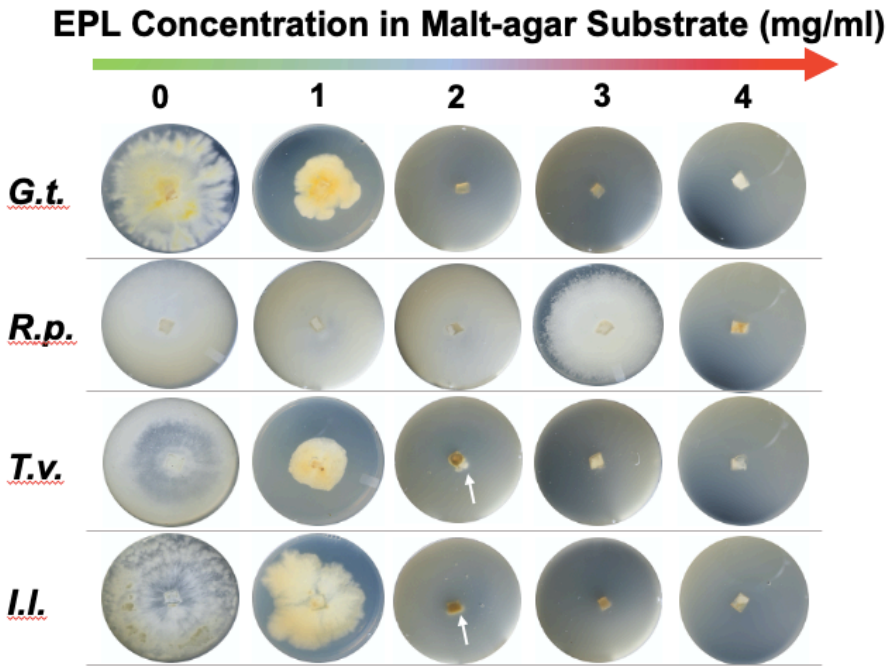

Figure 11. Photographs of four wood-decaying fungi grew on malt-agar substrates amended with 0, 1, 2, 3 and 4 mg/ml of EPL on day 14. The arrows in the Petri dishes represent mycelia colonization.

Upon EPL treatment, the four fungal strains responded differently in EPL-amended agar substrates, with *T.v.* being the most affected, while *R.p.* the least sensitive to EPL at the concentration of 1 mg/ml. For example, at this concentration, the average fungal growth rates for *G.t., R.p., T.v.* and *I.l.* are 19%, 100%,15%, and 59%, respectively, on day 14. Furthermore, mycelial color changed with EPL concentration and became darker in *Gt. T.v.* and *I.l.* in 1mg/ml (Figure 11).

As the concentration of EPL increases to 2 mg/ml, growth of *G.t.* is completely suppressed while the growth of both *T.v.* and *I.l.* had initial growth at early stage and then towards regression at a growth rate of less than 0.5% on day 14 (Figure 12) . For example, *T.v.* and *I.l.* stopped growing on day 9 (Figure 12a) and 4 (Figure 12b), respectively, and at this stage the hyphal tips of both fungi appear normal. However, on day 14, a condensation of protoplasm were observed, which could be related to retroversion of the growth (Riquelme

et al., 2018). In contrast, *R.p.* was very aggressive at EPL of 2 mg/ml and its growth was not completely contained until EPL concentration reach to 4 mg/ml. Although EPL of 2 mg/ml completely or almost retard the growth of *G.t.*, *T.v.* and *I.l.*, some replicates from these fungi continued growing after two-month check-up of the Petri dishes (*i.e.* for *G.t.* 2 out of 5 continue growing, for *T.v.* all the five replicates continue growing and for *I.l., 4 out of 5 grows*). Similar phenomenon was also observed in *R.p.* (2/5 continue growing) under EPL of 4 mg/ml. It is worth noted that no obvious hyphal expansion was seen in *G.t.*, *T.v.* and *I.l.* at 3 mg/ml and *R.p.* at 5 mg/ml after two-month follow up examination. Based on our preliminary study (un-published), the minimal inhibitory concentration of EPL against all the fungi was determined to be 3 mg/ml, except for *R.p.*, which is 5 mg/ml. Similar research has been reported that *Rhodonia placenta* is least sensitive to different organic acid (Barbero-López et al., 2020).

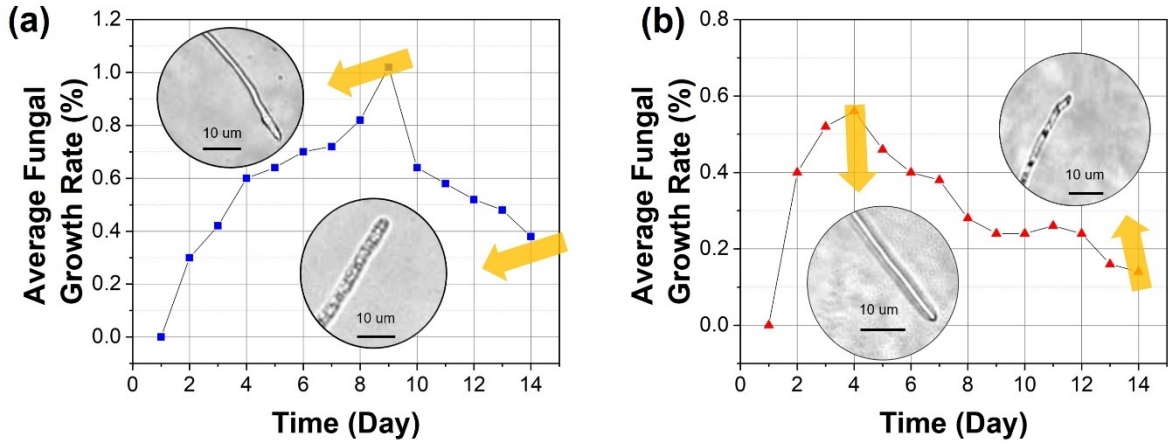

Figure 12. The growing curve and performance of *T.v.* (a) and *I.l.* (b) fungal hyphae after exposing 2mg/ml of EPL concentration under light microscopy. The *T.v.* (a) and *I.l.* (b) have highest growth rate at day 9 and day 4.

3.3.2 Microscopic Observations of Fungi Morphology due to the Effects of EPL

The morphology changes due to the effect of EPL were observed by microscopic observations, as shown in Figure 13. Significant differences of the hyphae growth between the control and EPL-amended malt agar were observed on all the fungal strains. In the control group, the hyphae display a smooth surface with tubular structure. On contrast, the hyphae of all the fungi grew on EPL-amended malt-agar substrates show twisted or distorted shape. The exposure of *R.p.* to EPL also triggers cell leakages. Similar research has been reported that when a fungus exposed to a fungicide, it will induce apoptotic-like, such as shrank, leakage, and rough surface (Chaves-Lopez et al., 2018; Hou et al. 2020).

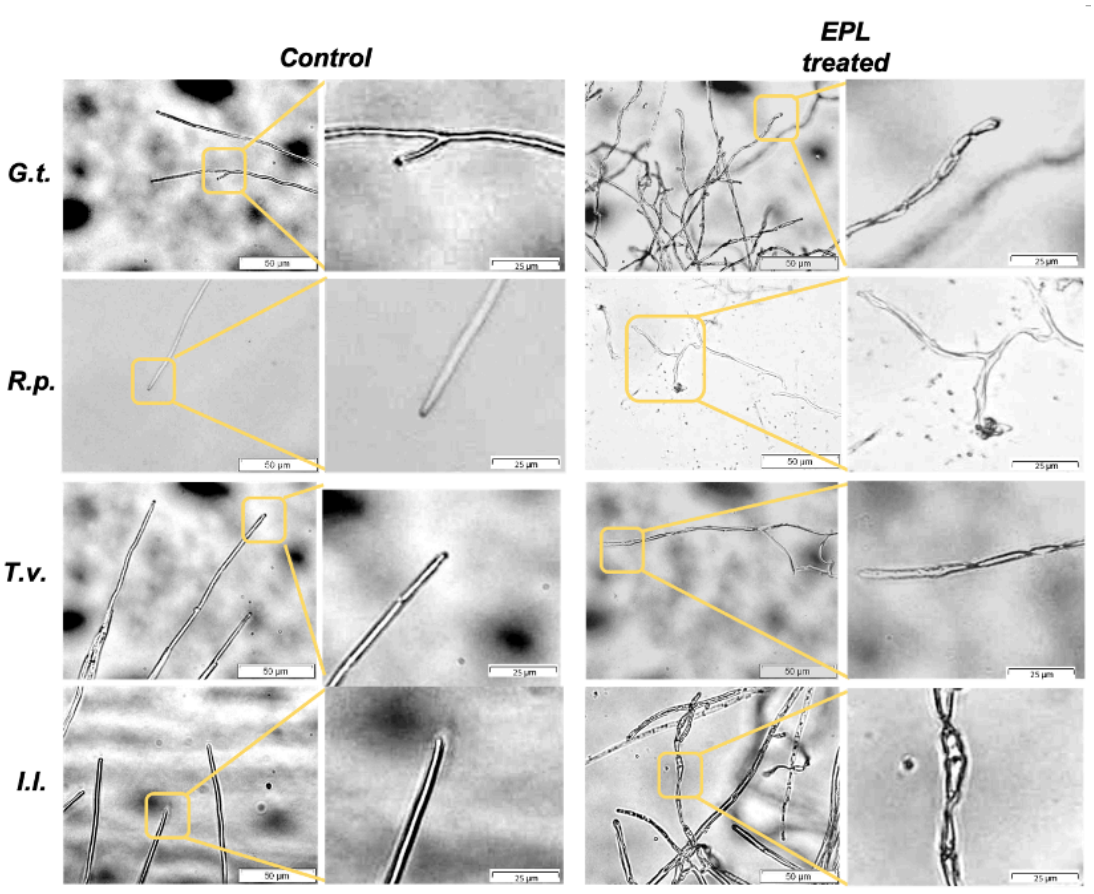

Figure 13. Optical micrographs showed morphological changes of the hyphae before and after the EPL treatments. The actively growing fungal tips were exposed to the control malt-agar substrate or amended with 1 mg/ml EPL for *G.t.,* 3 mg/ml EPL for *R.p.,* and 2 mg/ml EPL for both *T.v.* and *I.l.*.

Figure 14 shows that the fluorescence micrographs of the fungi exposed to control and EPL-amended malt-agar substrate on day 9. In the control group, intact nuclei were detected for the four fungi. In contrast, different fungi responded different to EPL treatment and led to various changes of nuclear. For example, in *G.t.*, the nuclei or debris of nuclei were not observed and were likely dissolved in the cytoplasm, indicating the high vulnerability of this specie exposure to chemicals (Thulasidas and Bhat, 2007). This observation is also consistent with the low growth rate of *G.t.* at EPL concentration of 1 mg/ ml. In comparison, part of the nuclei fragmented in the rest three fungal stains upon EPL treatment (Q. Gao et al., 2019) and some of them remains unchanged. These results could explain why *R.p.* and *I.l.* were less sensitive to EPL treatment at 1 mg/ ml. Although *T.v.* is very sensitive at 1 mg/ ml EPL treatment, the undamaged nuclear remained in the hyphal tips allow it to regrow more easily at EPL of 2 mg/ml after two-month check-in.

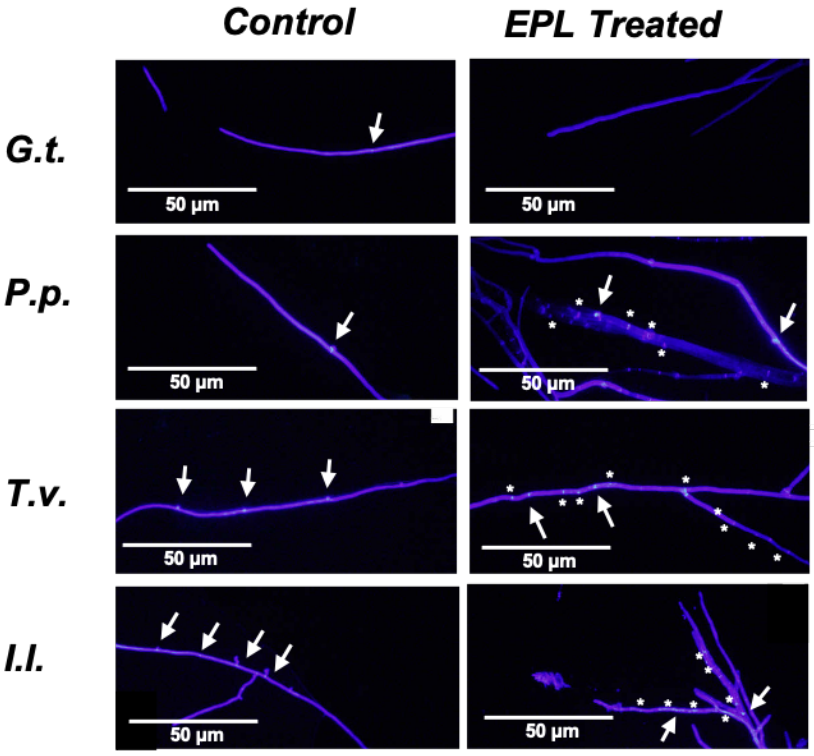

Figure 14. Fluorescent micrographs of four fungi, *G.t., R.p., T.v.* and *I.l.,* grew on EPL-amended malt-agar substrate at 1, 3, 2 and 2 mg/ml on day 9, respectively; un-amended malt-agar substrate was used as control. Intact nuclei were indicated by arrowheads (↑) while fragmented nuclei were indicated by *.

3.4 Conclusion

This study demonstrated the effectiveness of EPL against the common wood inhibiting fungal species on an agar substrate. Fungal species responses differed with different treatment amounts. The minimal inhibitory concentration of EPL against *G.t.*, *T.v.* and *I.l.* was determined to be 3 mg/ml while that of *R.p.* was 5 mg/ml. EPL treatments also affect the morphology of the hyphae by twisting and deforming their shapes. Future research will focus on testing the efficacy of EPL against wood-decaying fungi on and in wood.

Chapter 4: Epsilon Poly L-lysine (EPL) for Wood Protection

Abstract

Epsilon-Poly-L-Lysine (EPL) is produced by *Streptomyces albulus* strain 346 substance as a secondary metabolite. It has been used as an antimicrobial agent in food and medical fields. However, the application of EPL in the wood industry is currently unexamined. In this study, the decay resistance of EPL-treated wood at four different concentrations (1%, 10%, 15%, and 0% control) was studied by exposing to three fungal species, including one brown-rot fungus, *Rhodonia placenta (R.p.)*, and two white-rot fungi, *Trametes versicolor (T.v.)* and *Irpex lacteus (I.l.).*, The 1% EPL treatment reported the highest efficacy yielding low or negligible mass losses in decay test. The wood mass lost following an increase in treatment concentrations. The reason is because when the concentration of EPL increased, there was increased leaching from the wood, which resulted in higher mass loss. Additionally, EPL- treated wood had a high heat resistance. This study reports that the EPL could be an antifungal preservative and with thermal stability to improve wood durability.

4.1 Introduction

Wood preservation has been under development for many years (Ramage et al., 2017). The increasing needs of wood products, such as saw logs, cross-laminated timber, or veneers, triggered the development of methods for protecting wood against fungi, insects, radiation, fire, and moisture. Besides these considerations, if people can consider environmentally friendly purposes when producing wood preservatives for extending the wood service life, that will greatly decrease environmental hazards. The organic wood

preservatives are produced by organic chemicals which will lead to a clean environment (Binbuga, Ruhs, Hasty, Henry, & Schultz, 2008).

Epsilon-Poly-L-Lysine (EPL) is a nontoxic food preservative and was found around 30 years ago as the by-product of a Dragendorff-positive substance generated by isolating *Streptomyces albulus* strain 346 substance from soil (Shoji Shima & Sakai, 1977; Bankar and Singhal 2013). It is a high molecular weight compound with 25 to 35 identical l-lysine residues connected by ε-amino and α-carboxyl groups (Shima & Sakai, 1981; Shih, Shen, & Van, 2006). Shima and Shoji reported that the EPL is highly active against a broad spectrum of microorganisms, including fungi, bacteria, and specific viruses due to the electrostatic absorption mechanism (Shoji Shima, et al., 1984; Yoshida & Nagasawa, 2003). The NH_2 is a major amine group in EPL's backbone and can bind with negatively charged membranes from the microorganisms, thus disrupting the surface and cytoplasmic membrane (Yoshida & Nagasawa, 2003).

Currently, it has also been reported that the EPL has high-temperature resistance and can tolerance 20 minutes of autoclaving under 120°C or 30 minutes of boiling the solution under 100 °C (Yoshida & Nagasawa, 2003; Shih et al., 2006). It is also considered as a safe food preservative in many countries, such as Japan, Korea, and the United States (Yasuhiro et al., 2003). The US Food and Drug Administration has approved to use of it as a natural food preservative (USFDA, 2004). Because of these beneficial chemical properties, EPL has been widely applied in the medical and food preservation industries (Hyde et al., 2019). However, the application of EPL for wood protection has not been reported.

In this study, the antifungal activity of EPL against three wood-decaying fungi, including one brown-rot fungus, *Rhodonia placenta (R.p.)* and two white-rot fungi, *Trametes*

versicolor (T.v.) and *Irpex lacteus (I.l.),* were investigated in vivo. The retention of EPL in wood was measured and the mass loss to due fungal decay was also reported.

4.2 Methods and Materials

4.2.1 Materials

Epsilon Poly L-lysine (EPL, >95%, MW is between 3600 and 4300) was purchased from MarkNature (Qingdao, China). Agar, yeast-extract powder and malt extract were obtained from Fisher Chemical.

One brown-rot fungus, *R.p.* and two white-rot fungi*, T.v.* and *I.l.,* were donated by Dr. Juliet D. Tang at the USDA Forest Service Forest Products Laboratory, Starkville, MS and were used throughout the study.

Clear sapwood of southern pine (*Pinus spp*.) and poplar (*Populous tremuloides* Michx.) were used for brown-rot fungi for white-rot fungi decay tests, respectively. They were cut into 14 mm x 14 mm x 14 mm blocks for wood treatment and 3 mm x 28 mm x 34 mm as fungal feeder strips. Wood density was determined by a conventional method to calculate the ratio between oven-dry mass divide by the green volume of the same sample. The density of the wood samples after oven-drying were 491 kg/m^3 (SD 17, n=96) and 474 kg/m^3 (SD 38, n=48) for poplar and pine, respectively.

4.2.2 Characterization of Epsilon Poly L-Lysine (EPL) and EPL-treated wood

Three samples, EPL, untreated wood and EPL-treated wood (15 % EPL treatment on southern pine), were analyzed by using Fourier Transform Infrared Spectroscopy (FTIR, Nicolet IS 10, Thermo Scientific, Waltham, MA) and thermogravimetric analysis (TGA 7, Perkin Elmer, Waltham, MA). The pure EPL were directly used for the analysis. Untreated

wood and EPL-treated wood samples were treated with deionized water and 15% EPL treatment, respectively. After treating, EPL-treated wood samples were dipped into deionized water three times to remove free EPL on the surface of the wood. Both untreated wood and EPL-treated wood samples were oven-dried (60 ^{0}C, 48 H) and scratched into pellets from the surface of wood samples and placed in the 15ml centrifuge tubes. Centrifuge tubes were sealed with parafilm and drilled three tiny holes for moisture evaporation. After then all the samples (EPL, untreated wood, EPL-treated wood) were freeze-dried using a freeze drier (Labconco, USA) for another 48 h.

Specifically, to understand the changes associated with adding EPL to wood samples, the chemical changes of EPL treated wood were measured using FTIR The samples were collected in the wavenumber range of 400 to 4000 cm^{-1} at a resolution of 4 cm^{-1}. The obtained spectra were baseline corrected and normalized by using Omnic software.

The thermal stability of EPL treated wood (southern pine) was studied by using TGA instrument under nitrogen. 5 mg of EPL, untreated wood, and EPL-treated wood samples were placed on the platinum crucible and heated from room temperature to 800 ^{o}C at a heating rate of 20 ^{o}C/min, respectively (Lin, et al., 2018). Each sample was analyzed twice to collect the average for further analysis.

4.2.3 Preservatives treatment by impregnation of wood in EPL solutions

All the treatments are in 6 replicates. The total number of wood samples were 6 replicates x 4 EPL concentrations (3 treatments and one control) x 3 fungi (*R.p., T.v.* and *I.l.)* x 2 (w/ leaching and w/o leaching) = 144 replicates. All wood samples were immersed in the treating solutions and were subjected to vacuum (1000 mbar) for 20 min, followed with

atmospheric pressure for 30 min. This procedure was repeated for three times to ensure fully impregnation of EPL in wood. EPL concentrations of 1%, 10%, and 15% were included. The control cubes were impregnated with deionized water.

4.2.4 Quantification of EPL in wood by retention and mass gain

After impregnating process, the wet mass of the wood cube was recorded. The surplus solution was wiped from the surface of the cubes, and cubes were oven-dried at 60^0C for 48 h. EPL retention and mass gain were calculated according to the equations below:

$$\text{Retention } (kg/m^3) = \frac{GC}{V} \times 10$$

Where G represents mass difference between the wet mass of the samples after impregnation and their oven-dried before treatment (g), C means concentration of preservative solution (%), and V is volume of wood cubes in (cm^3).

$$\text{Mass Gain } (\%) = \frac{(M_{A-tret} - M_{B-tret})}{M_{B-tret}} \times 10$$

Where M_{B-tret} and M_{A-tret} are the oven-dried mass of wood samples before and after treatment, respectively.

4.2.5 Fluorescence microscopic analysis of treated wood

The micro-slides of wood samples in cross-sections were cut by a microtome (AO Spencer No. 860) (Patel et al., 2013). The angle of the blade (knife) was adjusted to 30^o to avoid distorting the wood structure during the cutting process (Richards, 1942). The thickness of these samples was 30 μm. The wood slices were mounted on the microscope slide with deionized water and were examined using fluorescence microscopy (BX 51/52 Olympus) under the blue (BA 420 nm), red (BA 515 nm), and green (BA 590 nm) light to

observe the distribution of EPL inside the wood. Then the three images were composited into one by using ImageJ software (Bankhead, 2014).

4.2.6 Leaching of impregnated wood samples

Before the wood durability test, wood cubes were subjected to a leaching test according to AWPA E11 standard to determine the leachability of wood preservatives. It can be used to predict potential environmental impacts of treated wood in service. Wood samples in the beakers were shaked at room condition with a speed of 100 rpm. The leachates were replaced with new deionized water after the time intervals of 1, 6, 24, and 48 h, thereafter every 48 h until a total leachate collection of nine were obtained. After leaching test, the wood cubes were removed from the beaker and the excess water on the cubes was wiped off. Finally. the wet mass and oven-dried mass after leaching test were recorded.

The amount of water needed for leaching test was calculated at the 1:2.5 (cube to water) ratio using the following equation:

$$\text{Water needed for impregnation (ml)} = V \times N$$

Where V represents the volume of wood samples, N represents the total number of cubes in each beaker for the leaching test. In this study, the volume of the cubes was 14*14*14 mm, and 12 cubes needed for leaching test. The total amount of water needed was 81 ml for leaching test.

4.2.7 Fungal resistance of treated wood

4.2.7.1 Preparation of feeder stripes

Wood feeder strips were pre-inoculated with either brown rot or white rot in the malt-agar substrate before durability test. Briefly, the malt-agar solution was formulated to consist

of malt extract, agar and yeast extract at a concentration of 2, 1.5 and 0.2%, respectively, using DI water as a solvent, followed by autoclaving at 120 ºC for 20 min. The molten malt-agar solution was transferred to the petri dishes (diameter of 90 mm) with sterilized syringes, of which 12.5 ml were cast per plate. After the malt-agar solution solidified, two pieces of same wood species feeder strips were placed on the malt agar separately. The total number of feeder strips were 6 replicates x 4 EPL concentration (3 treatments and one control) x 3 fungi (*R.p., T.v.* and *I.l.)* x 2 (w/ leaching and w/o leaching) = 144 replicates.. Then an active growing fungus plug with 20 mm in length were laid half on the feeder strip and half on the malt agar to confirm the fungi captured the nutrient from the feeder strips with active status. Finally, the petri dishes were sealed with parafilm and kept in a controlled chamber in the dark at a temperature of 25ºC and relative humidity of 75% until the fungal mycelium had fully covered on the surface of feeder strips.

4.2.7.2 Wood decay test

The antifungal efficacy of EPL treatments was determined by two-month exposure of southern pine and poplar to brown-rot and white-rot fungi, respectively, per AWPA E-10 standard. The feeder strips with active fungal growth were transferred to new culture bottle (malt-agar substrate). All wood cubes (144 pieces) with and without leaching test were sterilized by 70% of alcohol in the bio-safety hood for 30 minutes before inoculating to the culture bottles. The petri-dishes were sealed with parafilm and maintained at the conditioning chamber at the temperature of 25ºC and relative humidity of 75% for eight weeks.

After eight-week incubation, the samples were removed and the fungal mycelium on the surface of the wood cubes was brushed to obtain wet mass and oven-dried mass. These two numbers were used to calculate wood mass loss by fungi degradation. The equation was:

$$\text{Moisture content (\%)} = \frac{(w_{A-} - w_B)}{M_B} \times 100$$

Where w_A represents wet mass after being exposed to fungi, and M_B denotes oven-dried mass after being exposed to fungi.

$$\text{Mass loss (\%)} = \frac{(M_{B-f} - M_{A-f})}{M_{B-f}} \times 100$$

Where M_{B-f} and M_{A-f} are the oven-dried mass before and after being exposed to the wood-decaying fungi, respectively.

4.2.8 Statistical analysis

The data of retention, mass gain and mass loss were subjected to statistical analysis using SAS software (version 9.4; SAS Institute, Cary, North Carolina, USA). These include normality test, homogeneity of variance test and appropriate analysis of variance (ANOVA) method. For all our data, the homogeneity of variance is unequal, so Games-Howell test were used to compare all of group difference. The confidence level was set at 95%.

4.3. Results and Discussion

4.3.1 Fourier transform infrared spectroscopy (FTIR) and thermogravimetric analysis (TGA) of EPL

The FTIR spectra of EPL, wood, and EPL-treated wood are shown in Figure 15. In the ATR-FTIR spectrum of EPL, the band at 3206 cm^{-1} was determined as asymmetric stretching vibration of primary compound NH_2 (Maeda, et al., 2011). The band at 2859 cm^{-1} was characteristic of the C-H stretching vibration (Mathew & Abraham, 2008) . The band at 1553 cm^{-1} was the NH bending (amide II), and a band at 1657 cm^{-1} was described to the

carbonyl groups (C=O) stretching vibration (amide I). The C-N stretching of amide bands was observed at 1250 cm^{-1} (Hong, et al., 2011).

The spectra of wood specimen showed a broad band at 3337 cm^{-1} corresponding to the O-H bond in cellulose and hemicellulose (3000-3560cm^{-1}) (Esteves, et al., 2011) and a C-H stretching absorption at 2873 cm^{-1}. The 1732 cm^{-1} band was assigned to C=O in hemicelluloses, and C=C at 1505 cm^{-1} for aromatic in lignin. Whereas, the band at 1026 cm^{-1} assigned for C-O stretching in cellulose and hemicellulose(Pandey & Theagarajan, 1997).

After EPL treatment with wood, the NH_2 bending bands from EPL were overlapped with wood and decreased from wood at 3337 cm^{-1} to EPL-treated wood at 3324 cm^{-1}. The absorbance band of C=O at 1657 cm^{-1} from the EPL shifted to 1663 cm^{-1} in EPL-treated wood, which attributed to the formation of hydrogen bonds on carbonyl groups after crosslinking (C. Gao et al., 2014). Additionally, the C-H stretching vibration of wood at 2873 cm^{-1} shifted to 2866 cm^{-1}in EPL- treated wood, indicating there were interactions between the hydroxyl groups of wood (2873 cm^{-1}) and EPL's amino groups (3206 cm^{-1}).

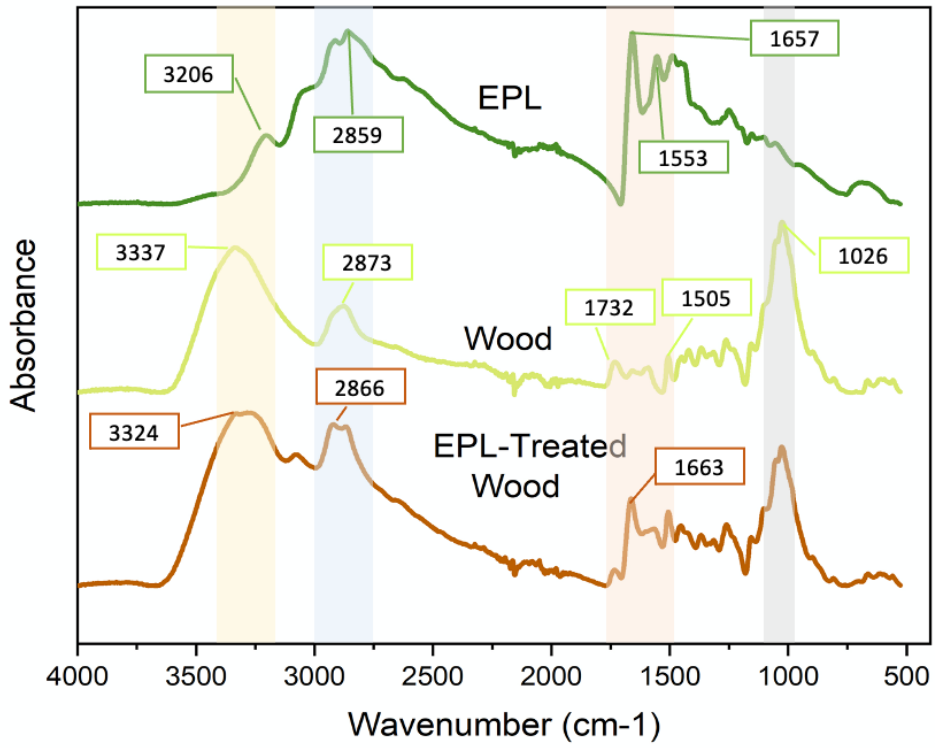

Figure 15. Infrared spectra of EPL, wood, and EPL-treated wood.

4.3.2 Thermogravimetric analysis

Figure 16 represented the thermogravimetric analysis (TGA) and derivative thermogravimetry (DTG) curves of EPL, wood, and EPL-treated wood in southern pine species. Overall, the degradation of mass loss were observed in EPL and EPL-treated wood while two were found in wood at room temperature to 800°C. At first stage (40 to 100^0C), the weight loss of EPL, wood, and EPL-treated wood were 3.8%, 4.9% and 7.0%, respectively. All the three treatments were decreased gradually at temperature from 40 to 100^0C, which is related to the release of moisture (Zhang et al., 2015).

The EPL sample showed good thermal stability from room temperature to 200^0C with a weight loss of 3.8% (Lin et al., 2018). It further decomposed dramatically at 200 to 500^0C

with peak at 328^{0}C and 448^{0}C due to stage 2 and 3, respectively (Yuan et al., 2019). The weight loss for stage 2 (200 to 430^{0}C) and stage 3 (430 to 500^{0}C) are 55.5% and 30.0%, respectively. Additionally, the temperature from 100 to 200^{0}C for three samples were presented more steady due to the release of noncombustible gases and liquids such as, acetic acid, CO_2, and H_2O (Dietenberger & Hasburgh, 2016).

The maximum weight loss (74.8%) of wood was found at second degradation stage (200^{0}C to 450^{0}C) with the highest degradation rate at 380^{0}C. In comparison, the EPL-treated wood showed lower thermal stability at beginning of thermal process than wood and EPL. At second stage of maximum mass loss point of EPL-treated wood (356^{0}C) was even lower than wood (380^{0}C) (Cruz et al., 2013). However, the huge change of curve was found in third stage. At this stage, the maximum mass loss point of EPL (448°C) was higher than wood (380°C), indicated EPL could tolerance higher thermal temperature. Therefore, the treatment of wood with EPL increase the thermal stability.

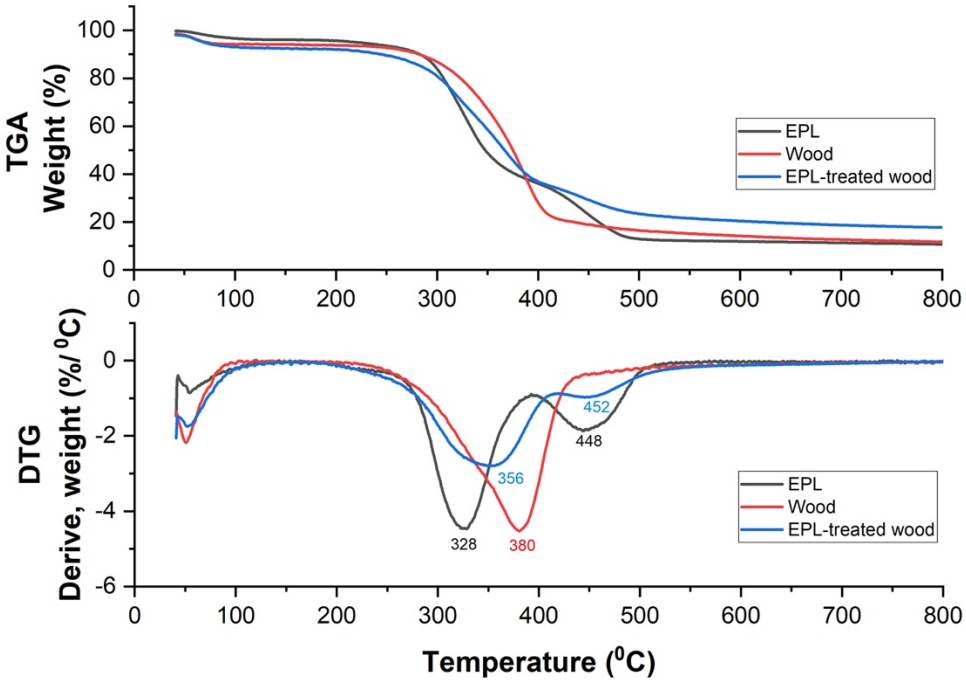

Figure 16. TGA and DTG thermograms of EPL, wood, and EPL-treated wood

4.3.3 EPL in wood

Retention and mass loss are two main factors used to quantify the amount of chemicals in wood after preservatives treatment. Specifically, retention is the one of the most convenient indicators to estimate the amount of preservatives in wood by calculating the differences between the mass of the untreated samples at room condition and the wet mass after treatment (Svedberg & Lindström, 2012). Mass gain can accurately quantify the amount of preservative absorbed by the wood after preservative treatment. It is obtained by calculating the oven-dried mass differences before and after treatment. This process is more tedious and could lead to the mitigation of the chemicals from inner part of the samples to the outer layer of samples (Dubey, Pang, & Walker, 2012).

Thus, retention is wide used in the wood industry (Svedberg & Lindström, 2012). In this study, both retentions and mass gain were used to quantify the EPL treatment effects (Figure 17) and end-matched samples were used throughout this study. Generally, retention and mass gain increased as a function of EPL concentration. For example, the retentions of EPL in hardwood at 1%, 10% and 15% were 8.75, 86.1, and 102.4 kg/m^{3,} respectively while in softwood at 1%, 10% and 15% were 7.54, 80.7, and 99.0 kg/m^3, respectively. Similar results were obtained in terms of mass gain. Also, based on the retention and mass gain (Figure 17) results, there were no significant differences between softwood and hardwood.

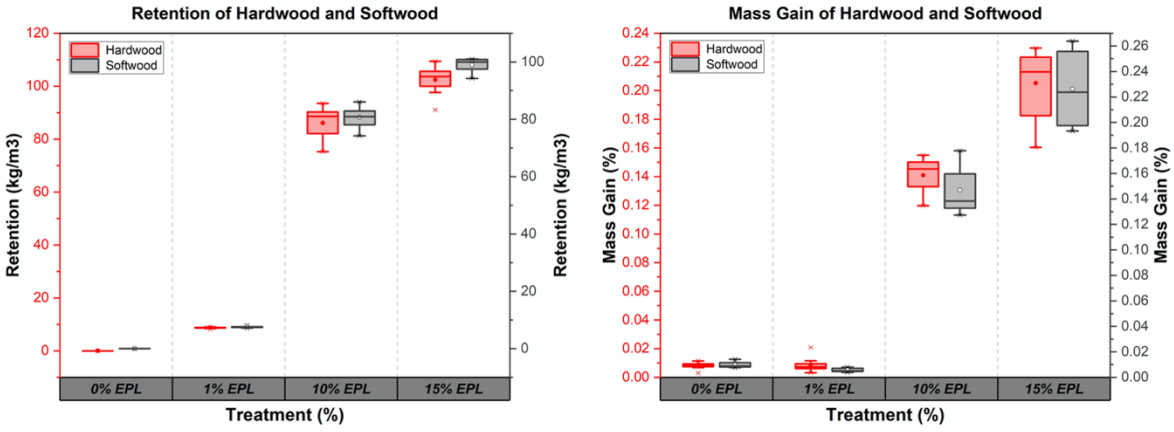

Figure 17. The Retention and mass gain of hardwood and softwood under different treatments.

The distribution of EPL along the cross-section of the wood samples under fluorescence microscopy is shown in Figure 18. The fluorescence microscopy images were presented in RGB format to maintain the original quality (Bankhead, 2014). Upon compositing, blue and purple colors was observed in the untreated wood (Echard et al., 2015) whereas red color was seen in the treated wood. This observation could be related to the different chemical molecules' energy states, emitting different light colors under

fluorescence microscopy (Vasileva et al., 2017; Drummen, 2012), indicating the existence of EPL in wood.

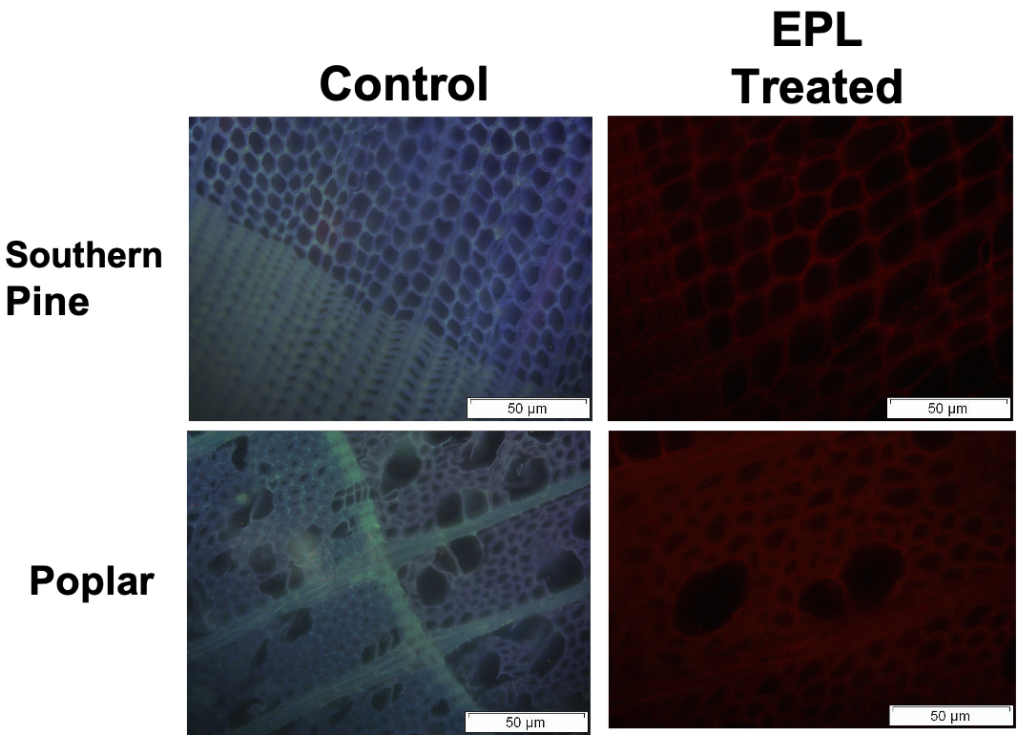

Figure 18. The composition of RGB (filter: BA 515 nm, BA 590 nm, BA 420 nm) light emitted on southern pine and poplar, treated with control and EPL treated under fluorescence microscopy.

4.3.4 Decay resistance of EPL against wood-decaying fungi

The photographs of all the samples before and after durability test are listed in Figure 19. As compared to control samples before fungal exposure and EPL treated samples after decay, significant damages were observed in the control groups after brown-rot decay. Whereas the appearance of white-rot decay cannot be easily visualized.

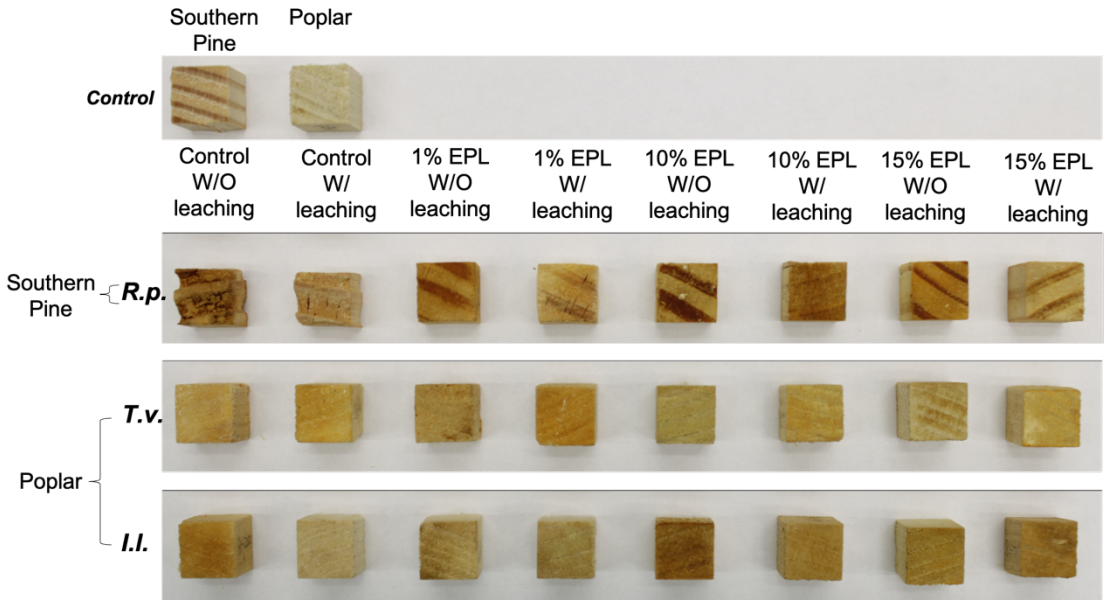

Figure 19. Photographs showing the wood cubes after eight weeks fungal exposure with different treatments.

Figure 21 shows a box and whisker plot of mass loss against different treatments. As can be seen, mass losses of the samples caused by fungal decay are significantly affected by EPL treatment. Especially, at EPL concentration of 1% and regardless of leaching test, the treated sample showed a significantly decreased mass loss for all the fungi tested, except for *T.v.*, as compared to the water-treated cubes. This means part of EPL has been removed during the leaching test and EPL remaining in wood was still effective against *R.p.* and *I.l.* but not against *T.v.*. The high mass loss of *T.v.*- exposed samples after leaching (28%) is possibly related to its partially undamaged nuclei, which allow *T.v.* to continue attacking wood, as shown in Figure 10 in Chapter 3. On the other hand, *R.p.* and *I.l.* may be more sensitive at a higher concentration of EPL exposure, even after leaching test.

As the concentration of EPL increased to 10% and 15 %, significantly higher mass losses were observed in all the fungal species in un-leached samples, which is related to the leaching of EPL (the yellowish color) during the durability test, as shown in Figure 20.

However, significantly lower mass losses were observed in the leached samples at high EPL concentrations as compared to those in the un-leached samples. This observation indicates that the remaining EPL in wood after leaching test can still provide adequate fungal resistance to these decaying fungi. The negative mass loss here is unknown and could be related to the residual hyphae mass in the wood after the durability test.

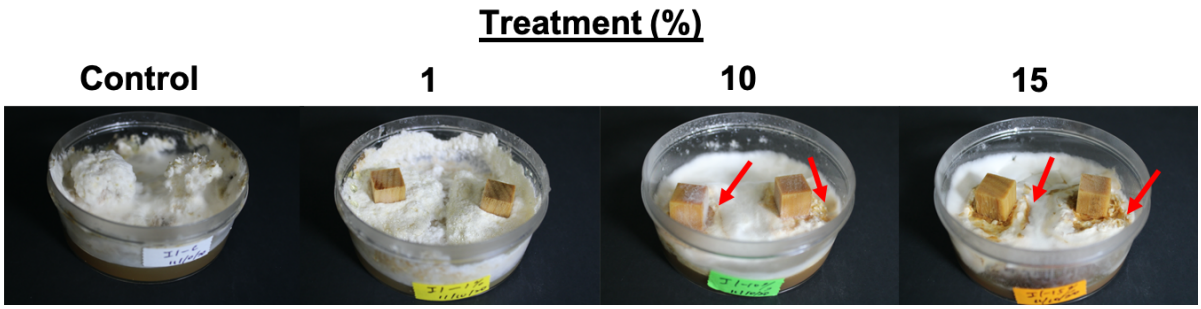

Figure 20. The culture containers showed un-leached poplar wood samples under wood durability test at different EPL treatments percentage. The red arrow indicated the EPL solution leached out at the EPL treatment at 10% and 15%.

Our 1% EPL treatment on wood (retention level of 7 kg/m^3) is comparable to current research findings by using ACQ and tannin-boron formulations (Tondi et al., 2012; Ung & Cooper, 2005). Upon further refinement of the formulation, it could be used for Use Category 4C: Ground contact or fresh water (American Wood Protection Association - AWPA, 2017).

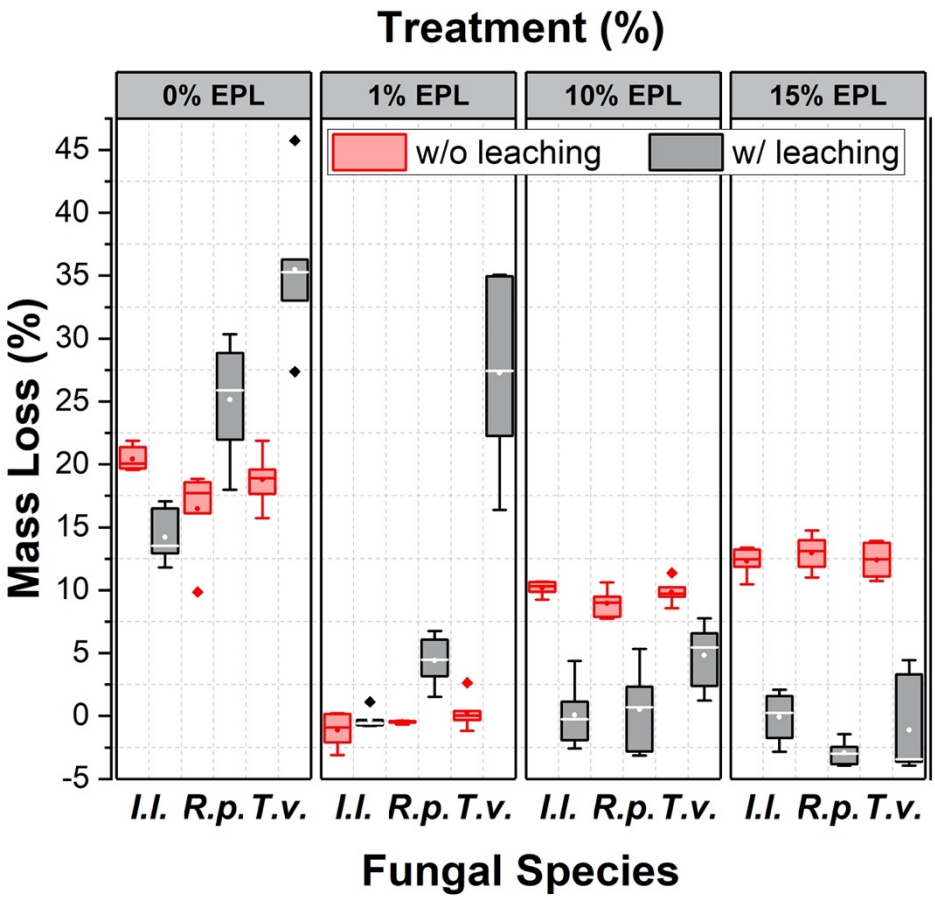

Figure 21. The mass loss percentage of three fungi performed at 1%, 10%, 15% and control (water treated) treatments.

4.4 Conclusion

This study has shown that the EPL as a wood preservative is an effective alternative for wood protection. The results suggest that the application of EPL should reach adequate-proportional quantity for preventing the leaching that causes high mass loss. The optimal percentage of EPL treatment for fighting against common wood-decaying fungi was determined to be 1%. Additionally, through TGA analysis, the EPL could resist heat when it is used to treat wood. Therefore, based on this preliminary-experimental result, the EPL could be proposed for broad application in wood preservatives.

Chapter 5: Conclusion

In North America, wood is a popular commodity with an important economic value. Unfortunately, they have a short service life when exposed to favorable environmental conditions of high relative humidity and temperature. The objective of this study was to test the feasibility of EPL for wood protection.

In the first part of the study, *in-vitro* tests of assessing the minimum inhibitory concentration of EPL to fight against *R.p.* were determined at 5 mg/ml and for *G.t., T.v.* and *I.l.* were determined at 3 mg/ml. Initially, all fungi hyphae were suppressed under the concentration at 4 mg/ml for *R.p*, while 2 mg/ml for *G.t., T.v.,* and *I.l.*. However, after a two-month checkup, the four fungal hyphae were slightly expanded at these concentrations (2 mg/ml and 4mg/ml), but not at 3 mg/ml for *G.t., T.v.*, and *I.l.,* and 5mg/ml for *R.p.*. The morphology of the fungal hyphae was modified upon EPL treatments, for example, twisting or deforming in hyphae were observed in all fungi and cell leakages were only seen in fungus *R.p.*.

Throughout the eight weeks of the wood durability test, the wood treated with 1% EPL has the least wood mass loss of -0.44% (without leaching test) and 10.20% (after leaching test) in average. These results compared to wood treated with 10% and 15% EPL treatments, it showed relatively lower wood mass loss percentage. For example, the 10% and 15% of EPL treatments showed the wood mass loss percentage was 10% and 12% at without leaching test, while 15% and 16% after leaching test, respectively. Furthermore, the 10% and 15% treatments of EPL without leaching test also showed higher mass loss than 1% treatment, indicating the EPL leached out during the incubation period. However, lower mass loss rates were obtained at 10% and 15% of EPL after the leaching test. The results addressed

that the remaining EPL compounds in the wood cubes could achieve adequate protection efficacy. Therefore, this study reported that an appropriate amount of EPL application was a critical requirement for protecting wood against common wood-decaying fungi. The thermal stability of EPL treated wood was also evaluated by TGA and we find that that EPL-treated wood could tolerate higher temperatures than the wood without EPL treatment.

Ensuring that EPL is effectively fixed in the wood cells without leaching out from wood cubes will be a priority for further experiments. In this preliminary study, EPL-treated wood was only exposed in the malt-agar substrates with stable and high moisture contents. However, wood in the actual application typically is applied in an even harsher environment, such as heavy rain, snow, and wind. Therefore, an alternative method of fixing the EPL in the wood cells could be applied by controlling temperatures under 120^0C but higher 60^0C or adding additives. This alternative method might be used to evaluate the potential fixation of EPL compounds in wood cells without leaching when exposed to a severe environment.

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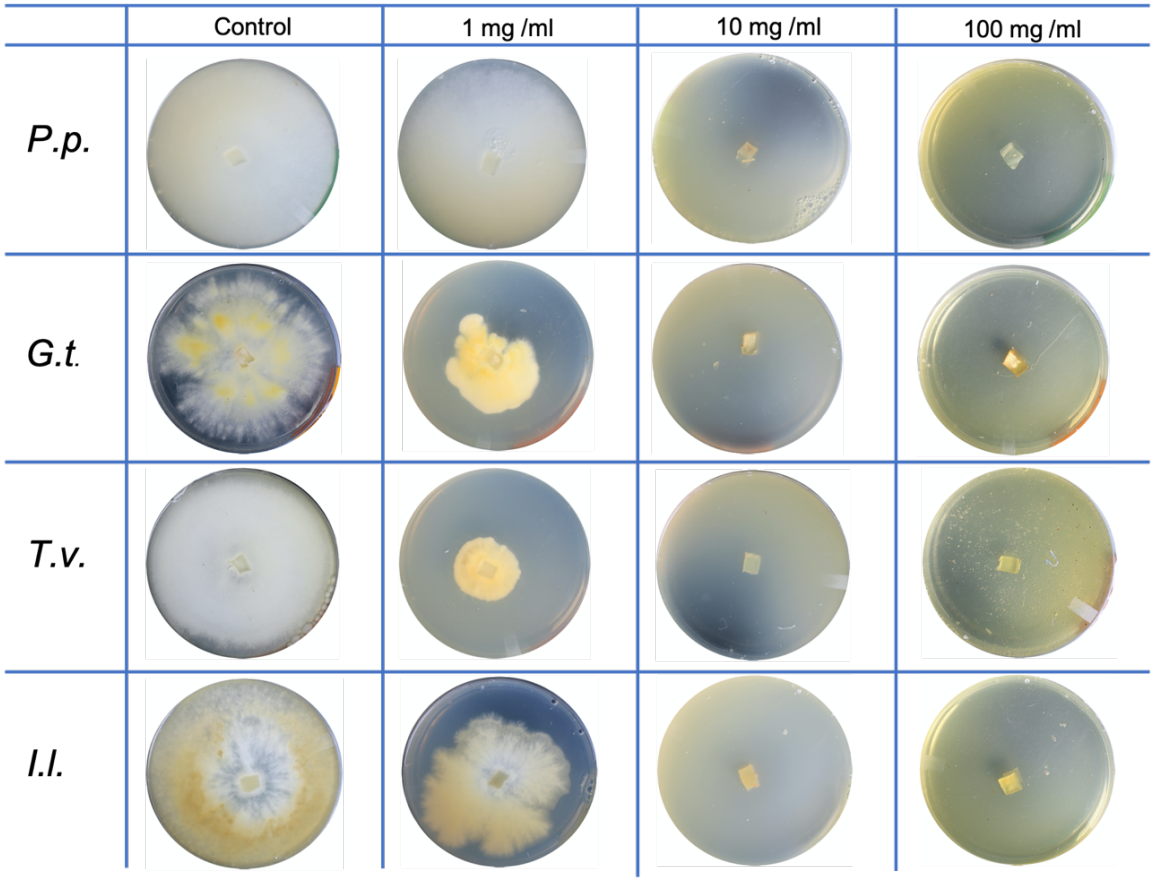
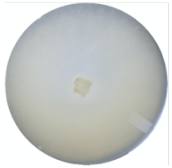
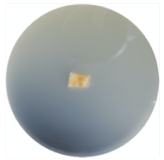
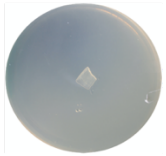
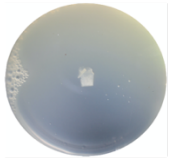
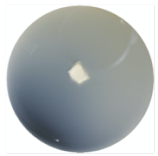
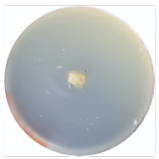
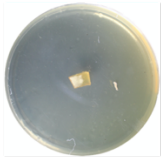
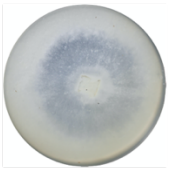
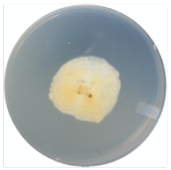
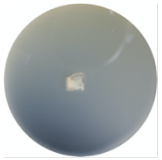
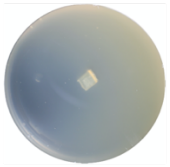
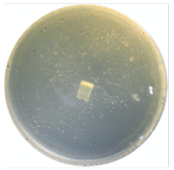
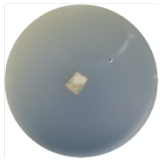
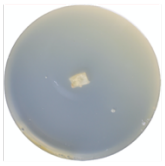
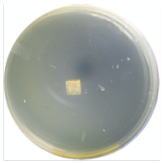
Appendices

The raw data of wood durability test related to formal and preliminary experiments.

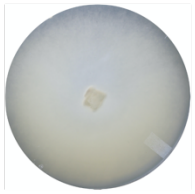
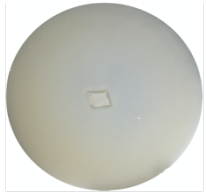
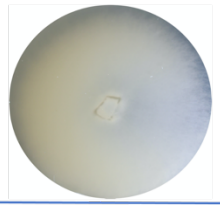
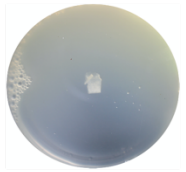
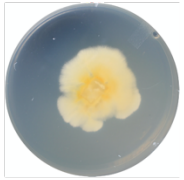
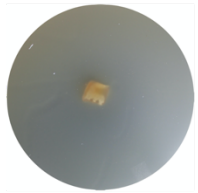
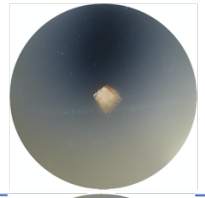
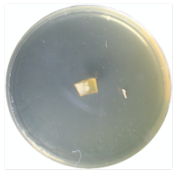
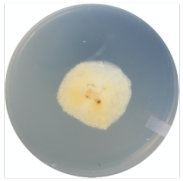
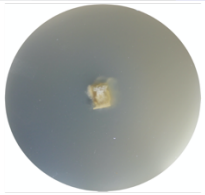
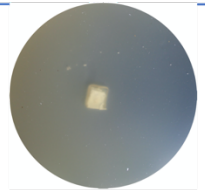
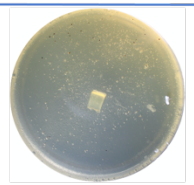
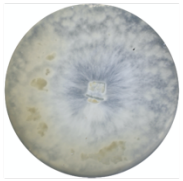
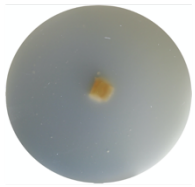
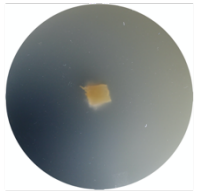
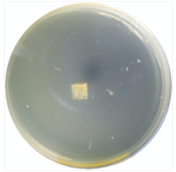
Appendix 1: The first- preliminary experiment of Epsilon Poly-L-lysine (EPL) treatments 1 mg, 10 mg, 100 mg at day 14th.



Appendix 2: The second preliminary experiment of Epsilon Poly-L-lysine (EPL) treatments, 1mg, 4mg, 7mg, 10mg at day 14th.

	Control	1 mg /ml	4 mg /ml	7 mg /ml	10 mg /ml
P.p.					
G.t.					
T.v.					
I.l.					

Appendix 3: The final determination of Epsilon Poly-L-lysine (EPL) concentration, 1 mg, 2mg, 3mg, 4mg at day 14th.

	Control	1 mg /ml	2 mg /ml	3 mg /ml	4 mg /ml
P.p.					
G.t.					
T.v.					
I.l.					

Appendix 4: Raw data of EPL fights against fungal growth rate

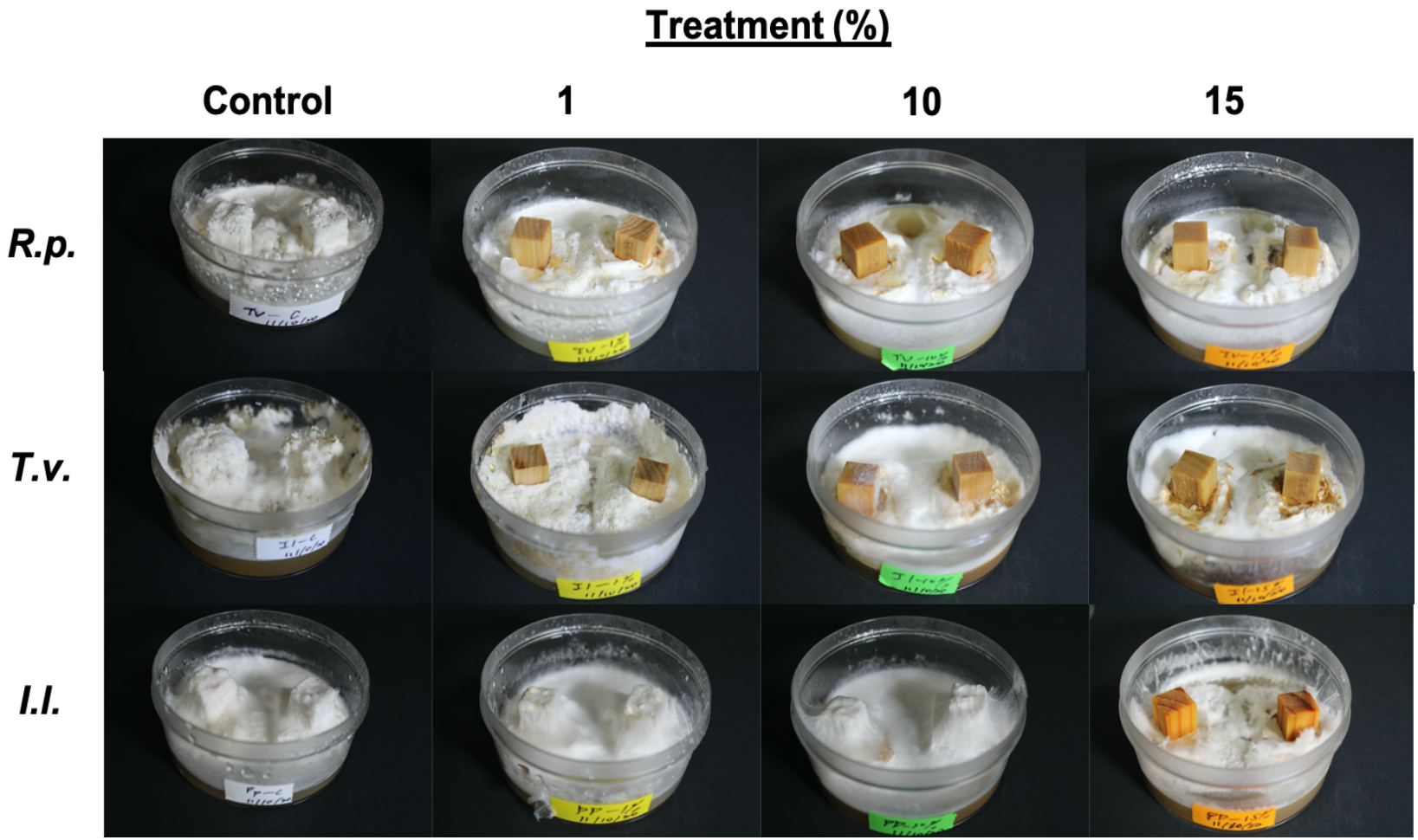
Treatment	Fungal species	Day 14 area	Day 13 Area	Day 12 Area	Day 11 Area	Day 10 Area	Day 9 Area	Day 8 Area	Day 7 Area	Day 6 Area	Day 5 Area	Day 4 Area	Day 3 Area	Day 2 Area	Day 1 Area
1 mg EPL	T.v.-1	925	798	774	653	616	539	469	406	393	298	274	210	163	130
1 mg EPL	T.v.-2	1215	1050	967	806	707	601	554	482	408	323	301	291	177	102
1 mg EPL	T.v.-3	1118	986	816	743	643	561	505	446	396	330	297	210	174	98
1 mg EPL	T.v.-4	963	843	748	677	561	485	432	390	322	300	245	180	153	125
1 mg EPL	T.v.-5	1078	904	749	723	597	522	451	384	314	274	230	168	131	95
1 mg EPL	I.a.-1	3683	3572	3445	3272	2996	2569	2403	1906	1748	1443	1067	778	265	101
1 mg EPL	I.a.-2	3871	3671	3470	3266	2887	2673	2397	2080	1779	1513	1144	907	331	129
1 mg EPL	I.a.-3	4084	3657	3615	3422	2922	2703	2251	2071	1775	1319	945	663	236	100
1 mg EPL	I.a.-4	4419	4405	4122	3900	3470	3259	2930	2700	2038	1670	1190	873	253	136
1 mg EPL	I.a.-5	3266	3247	3111	2940	2769	2480	2156	2081	1763	1493	1078	931	341	119
1 mg EPL	G.t.-1	1799	1540	1390	1270	1091	943	881	786	708	602	455	347	169	112
1 mg EPL	G.t.-2	1724	1474	1353	1127	955	858	654	571	464	348	308	237	155	108
1 mg EPL	G.t.-3	1174	1087	1051	1047	894	886	747	729	620	552	456	314	140	92
1 mg EPL	G.t.-4	1009	836	783	761	703	589	477	414	339	282	233	211	124	79
1 mg EPL	G.t.-5	772	722	721	742	696	683	644	618	599	553	481	344	130	85
1 mg EPL	P.p.-1	6896	6889	6888	6473	6370	5707	4022	3695	2096	1199	384	275	164	83
1 mg EPL	P.p.-2	6826	6806	6798	6497	6088	5600	4180	3199	2486	1072	485	335	196	97
1 mg EPL	P.p.-3	6891	6700	6494	6222	5581	5464	4520	3419	2837	1072	688	291	162	99
1 mg EPL	P.p.-4	6887	6841	6652	6619	6099	5939	4888	3799	3346	1850	799	353	239	125
1 mg EPL	P.p.-5	6840	6890	6473	6363	6095	5849	4479	3760	2553	1264	740	314	156	95

Treatment	Fungal species	Day 14 area	Day 13 Area	Day 12 Area	Day 11 Area	Day 10 Area	Day 9 Area	Day 8 Area	Day 7 Area	Day 6 Area	Day 5 Area	Day 4 Area	Day 3 Area	Day 2 Area	Day 1 Area
control	T.v.-1	6492	6496	6489	6481	6496	6488	6440	6441	6438	5950	4431	2776	530	127
control	T.v.-2	6481	6483	6482	6460	6474	6419	6499	6489	6431	6125	4588	3077	671	148
control	T.v.-3	6488	6488	6471	6446	6414	6461	6413	6493	6433	6012	4720	3037	588	156
control	T.v.-4	6430	6435	6449	6436	6439	6461	6421	6412	6411	6009	4472	3209	666	141
control	T.v.-5	6445	6436	6428	6455	6401	6417	6488	6430	6473	6182	4278	3110	569	135
control	I.a.-1	6439	6466	6450	6438	6427	6412	6421	6426	6477	6435	5387	4928	938	89
control	I.a.-2	6421	6490	6464	6496	6415	6461	6439	6426	6450	6483	5491	5019	1030	73
control	I.a.-3	6453	6450	6460	6492	6446	6440	6466	6444	6432	6477	5564	5147	1266	117
control	I.a.-4	6445	6421	6487	6467	6429	6445	6423	6489	6477	6408	5577	5279	1300	128
control	I.a.-5	6413	6421	6446	6417	6401	6435	6474	6452	6451	6449	5535	5184	1226	109
control	G.t.-1	5935	5700	5289	5116	4379	3863	3766	3057	2616	1889	1419	1053	302	114
control	G.t.-2	6285	6126	5969	5347	4735	4574	4436	3728	3227	2652	1990	1120	241	89
control	G.t.-3	6365	6026	5798	5674	5037	4111	3617	2980	2459	1838	1354	915	217	89
control	G.t.-4	6442	6465	5885	5802	5082	4392	4011	3357	2602	2583	1408	991	235	95
control	G.t.-5	6428	5773	5685	5599	5274	5199	5023	4308	3844	2274	1611	1092	229	89
control	P.p.-1	6476	6457	6430	6298	6190	5264	5201	4856	3706	2955	1750	917	171	112
control	P.p.-2	6456	6425	6358	6277	6141	5211	5137	4502	3863	3090	1594	797	157	76
control	P.p.-3	6123	5855	5812	5700	5243	5217	4936	4267	3899	3259	1928	1231	211	107
control	P.p.-4	6482	6475	6467	6322	6016	5755	5583	4585	4030	3611	2203	1413	221	95
control	P.p.-5	6485	6481	6433	6256	6156	5458	5293	4777	4373	3429	2134	1134	168	84

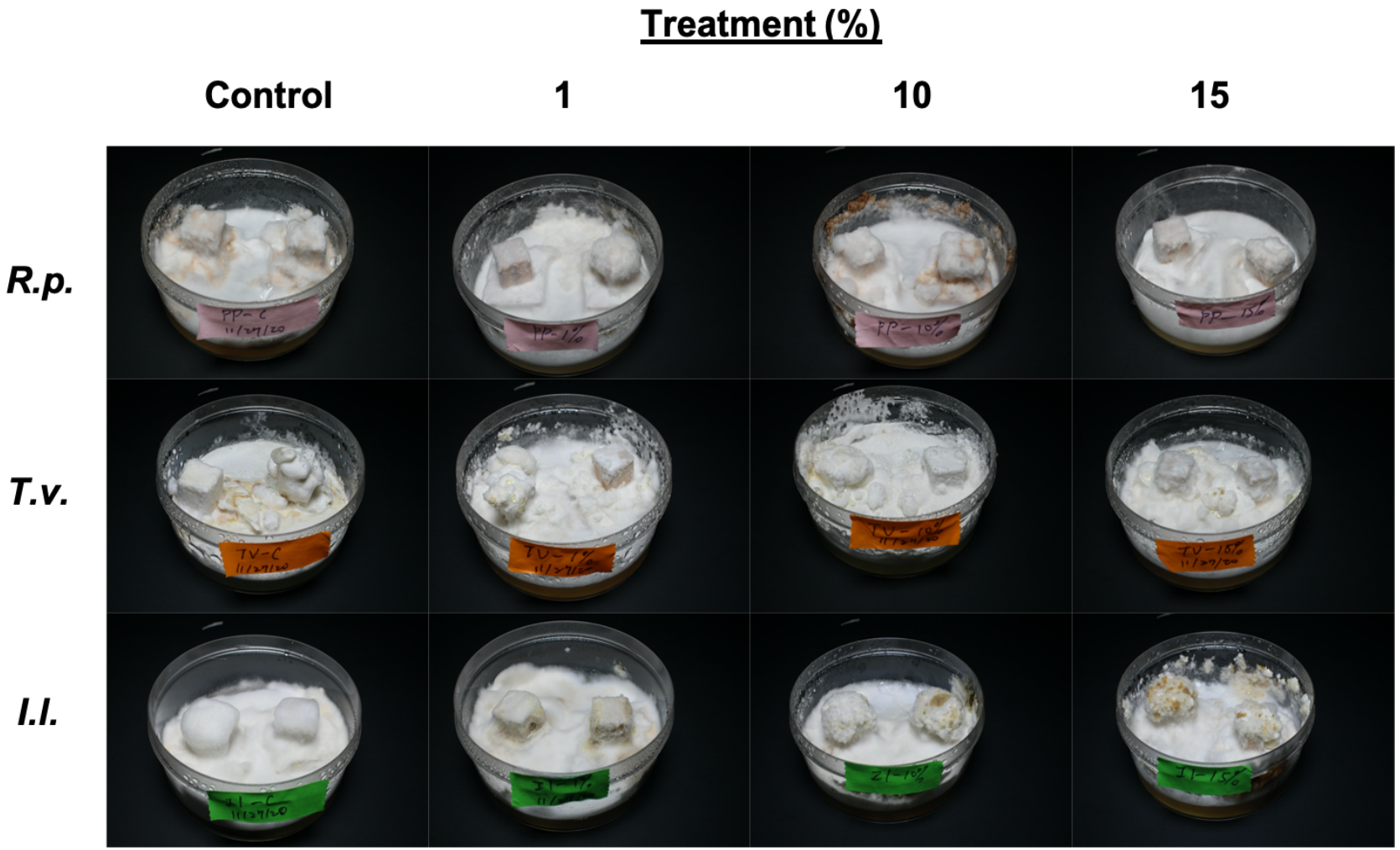
Treatment	Fungal species	Day 14 area	Day 13 Area	Day 12 Area	Day 11 Area	Day 10 Area	Day 9 Area	Day 8 Area	Day 7 Area	Day 6 Area	Day 5 Area	Day 4 Area	Day 3 Area	Day 2 Area	Day 1 Area
2 mg EPL	T.v.-1	79	78	85	89	90	113	112	107	102	100	98	97	92	77
2 mg EPL	T.v.-2	141	142	143	151	160	167	145	128	126	124	123	116	106	100
2 mg EPL	T.v.-3	93	96	98	101	105	120	116	115	114	112	104	98	91	71
2 mg EPL	T.v.-4	154	176	179	180	189	222	208	198	196	190	185	157	139	98
2 mg EPL	T.v.-5	77	76	78	81	85	115	106	100	99	97	96	93	89	73
2 mg EPL	I.a.-1	90	90	95	95	96	98	102	102	100	103	106	102	97	72
2 mg EPL	I.a.-2	88	88	97	97	98	96	97	98	112	118	127	112	107	86
2 mg EPL	I.a.-3	81	90	94	94	93	96	98	113	113	113	116	112	108	77
2 mg EPL	I.a.-4	100	100	101	101	103	102	103	103	103	109	124	124	121	88
2 mg EPL	I.a.-5	96	95	95	97	96	96	98	112	112	110	121	121	106	87
2 mg EPL	G.t.-1	N/A													69
2 mg EPL	G.t.-2														96
2 mg EPL	G.t.-3														85
2 mg EPL	G.t.-4														80
2 mg EPL	G.t.-5														82
2 mg EPL	P.p.-1	6885	6844	6847	5037	4166	2764	1564	879	515	279	188	110	96	52
2 mg EPL	P.p.-2	6820	6815	6736	5111	3669	2756	1538	813	536	222	156	134	117	73
2 mg EPL	P.p.-3	6819	6821	6801	4862	3627	2773	1793	1008	487	245	188	118	79	50
2 mg EPL	P.p.-4	6821	6802	6818	5663	4911	3348	2046	1265	703	331	215	133	112	76
2 mg EPL	P.p.-5	6877	6789	6702	4843	3590	2440	1832	985	614	312	197	108	83	82

Treatment	Fungal species	Day 14 area	Day 13 Area	Day 12 Area	Day 11 Area	Day 10 Area	Day 9 Area	Day 8 Area	Day 7 Area	Day 6 Area	Day 5 Area	Day 4 Area	Day 3 Area	Day 2 Area	Day 1 Area
3 mg EPL	T.v.-1	N/A													91
3 mg EPL	T.v.-2														106
3 mg EPL	T.v.-3														111
3 mg EPL	T.v.-4														104
3 mg EPL	T.v.-5														84
3 mg EPL	I.a.-1														72
3 mg EPL	I.a.-2														72
3 mg EPL	I.a.-3														69
3 mg EPL	I.a.-4														91
3 mg EPL	I.a.-5														92
3 mg EPL	G.t.-1														80
3 mg EPL	G.t.-2														91
3 mg EPL	G.t.-3														81
3 mg EPL	G.t.-4														75
3 mg EPL	G.t.-5														97
3 mg EPL	P.p.-1	6884	6806	6202	5304	4239.471	2955	2631	1588	829	312	219	150	102	91
3 mg EPL	P.p.-2	6807	6804	6233	4869	3421.176	1952	1604	766	381	173	131	130	84	75
3 mg EPL	P.p.-3	6887	6848	6226	5854	4836.14	3916	3120	2067	1327	451	381	243	103	100
3 mg EPL	P.p.-4	6873	6250	5624	4659	3667.362	2774	2529	1411	1063	449	357	215	101	92
3 mg EPL	P.p.-5	6761	6730	5418	5001	4018.418	3176	2537	1532	1198	498	366	248	104	102

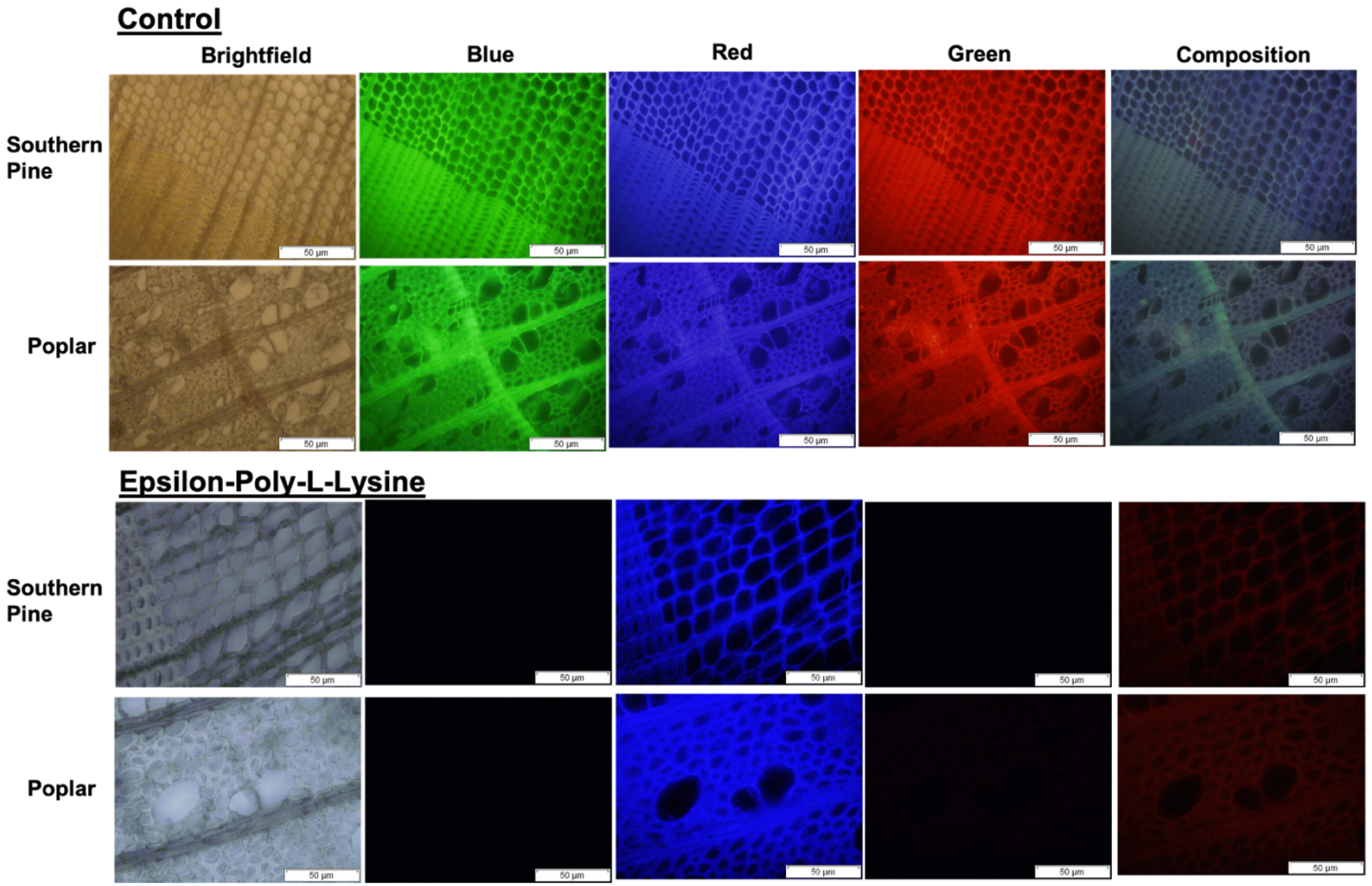
Appendix 5: Wood cubes after 8 weeks exposing to fungal culture bottle (without leaching test)



Appendix 6: Wood cubes after 8 weeks exposing to fungal culture bottle (with leaching test)



Appendix 7: The composition of RGB light on southern pine and poplar treated with control and EPL under fluorescence microscopy.



Appendix 8: Raw data of wood durability test (retention, mass gain, mass loss)

Treatment (trt)	wood species	Sample ID	Poplar's % of sapwood	Fungi exposed	Room Temperature Mass before trt (g)	Oven-dried mass before trt (m1)-- 1st trial	Oven-dried mass before trt (m1)-- 2nd trial	% of difference from 1st & 2nd Trial	Wet mass after trt (m2)	Retentions (kg/m3)	Oven-dried mass after trt (m3)---------- 1st trial	Oven-dried mass after trt (m3)---------- 2nd trial	% of difference from 1st & 2nd Trial	Mass gain %		Wet mass after leacheing	Oven-dried mass after Leaching---- 1st trial	Oven-dried mass after Leaching---- 2nd trial	Wet mass after fungi exposure (m4)	Oven-dried mass after fungi exposure (m5)---------- 1st trial	Oven-dried mass after fungi exposure (m5)---------- 2nd trial	% of difference from 1st & 2nd Trial	MC after decaying %	Mass loss %
Control	Hardwood	3-1		T.v.	1.497	1.4432	1.4467	0.2%	3.8585	0.0000	1.4348	1.4329	0.1%	1%	non-leaching				2.4543	1.1615	1.1597	0.2	111.3	19.05
		3-2		T.v.	1.4777	1.4285	1.4314	0.2%	3.7987	0.0000	1.4150	1.4130	0.1%	1%					1.9931	1.1053	1.1041	0.1	80.3	21.89
		3-3		T.v.	1.4194	1.3621	1.3690	0.5%	3.8009	0.0000	1.3580	1.3563	0.1%	1%					2.6125	1.1027	1.1021	0.1	136.9	18.80
		3-7		T.v.	1.4894	1.4346	1.4363	0.1%	3.8358	0.0000	1.4228	1.4208	0.1%	1%					2.2417	1.1442	1.1437	0.0	95.9	19.58
		3-9		T.v.	1.3885	1.3329	1.3397	0.5%	3.8057	0.0000	1.3294	1.3278	0.1%	1%					2.3334	1.1201	1.1204	0.0	108.3	15.74
		3-11	20	T.v.	1.3012	1.2512	1.2595	0.7%	3.6511	0.0000	1.2500	1.2488	0.1%	1%					2.2429	1.0293	1.0287	0.1	117.9	17.66
		3-12		T.v.	1.4319	1.3892	1.3863	0.2%	3.8948	0.0000	1.3726	1.3711	0.1%	1%	leaching	3.0563	1.3676	1.3672	1.4284	0.7420	0.6959	0.1	92.5	45.94
		3-13	30	T.v.	1.3546	1.3216	1.3139	0.6%	3.7651	0.0000	1.3050	1.3037	0.1%	1%		2.8375	1.2990	1.2988	2.0093	0.8353	0.7888	0.1	140.5	35.99
		3-14	30	T.v.	1.3006	1.2622	1.2598	0.2%	3.6357	0.0000	1.2505	1.2484	0.2%	1%		2.7141	1.2453	1.2444	3.0830	0.9045	0.8987	0.0	240.9	27.67
		3-15	70	T.v.	1.4224	1.3729	1.3718	0.1%	3.8346	0.0000	1.3598	1.3584	0.1%	1%		3.0278	1.3532	1.3530	1.3975	0.8812	0.8355	0.1	58.6	35.20
		3-16	80	T.v.	1.4088	1.3602	1.3627	0.2%	3.8454	0.0000	1.3524	1.3507	0.1%	1%		2.8969	1.3458	1.3455	1.5154	0.9015	0.8552	0.1	68.1	33.34
		3-18		T.v.	1.367	1.3301	1.3232	0.5%	3.7204	0.0000	1.3115	1.3096	0.1%	1%		2.9633	1.3052	1.3048	1.8765	0.8314	0.7866	0.1	125.7	36.61
		3-19		I.l.	1.4408	1.3896	1.3957	0.4%	3.7558	0.0000	1.3838	1.3822	0.1%	1%	non-leaching				2.3781	1.0880	1.0861	0.2	118.6	21.38
		3-20	20	I.l.	1.3061	1.2611	1.2681	0.6%	3.6832	0.0000	1.2592	1.2571	0.2%	1%					2.2581	1.0086	1.0079	0.1	123.9	19.90
		3-22		I.l.	1.521	1.4779	1.4777	0.0%	3.9214	0.0000	1.4639	1.4617	0.2%	1%					2.5330	1.1680	1.1698	0.2	116.9	20.21
		3-23	40	I.l.	1.2996	1.2631	1.2635	0.0%	3.6650	0.0000	1.2537	1.2518	0.2%	1%					2.3226	1.0070	1.0063	0.1	130.6	19.68
		3-24		I.l.	1.4397	1.3939	1.3991	0.4%	3.8024	0.0000	1.3860	1.3842	0.1%	1%					2.4104	1.1149	1.1131	0.2	116.2	19.56
		3-25		I.l.	1.453	1.4234	1.4129	0.7%	3.8502	0.0000	1.3996	1.3973	0.2%	1%					2.6067	1.0932	1.0941	0.1	138.4	21.89
		3-26		I.l.	1.5235	1.4914	1.4803	0.7%	3.9047	0.0000	1.4661	1.4629	0.2%	1%	leaching	3.1243	1.4593	1.4586	2.4455	1.2185	1.2113	0.0	100.7	16.89
		3-28	10	I.l.	1.3542	1.3214	1.3102	0.8%	3.7517	0.0000	1.3010	1.2984	0.2%	1%		2.8013	1.2922	1.2919	1.9974	1.1111	1.1156	0.0	79.8	14.60
		3-29		I.l.	1.5177	1.4683	1.4686	0.0%	3.9452	0.0000	1.4560	1.4536	0.2%	1%		3.1123	1.4496	1.4485	2.4800	1.2021	1.2066	0.0	106.3	17.44
		3-30	80	I.l.	1.324	1.2822	1.2838	0.1%	3.6436	0.0000	1.2797	1.2768	0.2%	0%		2.7892	1.2724	1.2725	2.2095	1.1077	1.063	0.0	99.5	13.44
		3-31		I.l.	1.4174	1.3884	1.3806	0.6%	3.7976	0.0000	1.3678	1.3648	0.2%	1%		3.0017	1.3609	1.3612	2.1625	1.2001	1.1995	0.0	80.2	12.26
		3-32		I.l.	1.4004	1.3474	1.3553	0.6%	3.8524	0.0000	1.3512	1.3484	0.2%	0%		3.0072	1.3454	1.3448	2.1175	1.1701	1.1691	0.0	81.0	13.40
	Softwood	2-13		P.p.	1.2879	1.2562	1.2517	0.4%	3.4286	0.0000	1.2418	1.2391	0.2%	1%	Non-leaching				3.1897	1.0077	1.0101	0.2	216.5	18.85
		2-14		P.p.	1.2661	1.2333	1.2313	0.2%	3.4290	0.0000	1.2209	1.2189	0.2%	1%					3.2356	1.0010	1.0081	0.7	223.2	18.01
		2-15		P.p.	1.2310	1.2013	1.1978	0.3%	3.3436	0.0000	1.1887	1.1858	0.2%	1%					2.9327	0.9815	0.9789	0.3	198.8	17.43
		2-16		P.p.	1.2701	1.2323	1.2380	0.5%	3.4053	0.0000	1.2281	1.2258	0.2%	1%					3.2445	1.0302	1.0321	0.2	214.9	16.11
		2-17		P.p.	1.2054	1.1840	1.1750	0.8%	3.3467	0.0000	1.1653	1.1631	0.2%	1%					2.9161	1.0504	1.04162	0.8	177.6	9.86
		2-18		P.p.	1.2856	1.2543	1.2574	0.2%	3.4422	0.0000	1.2418	1.2395	0.2%	1%					3.2701	1.0111	1.0091	0.2	223.4	18.58
		2-19		P.p.	1.3385	1.3089	1.2998	0.7%	3.4758	0.0000	1.2877	1.2849	0.2%	1%	leaching	2.9474	1.2816	1.2816	1.7990	1.0000	0.9982	0.0	79.9	22.34
		2-20		P.p.	1.3026	1.2542	1.2585	0.3%	3.4727	0.0000	1.2450	1.2422	0.2%	1%		2.9029	1.2380	1.2382	2.1224	0.9043	0.9098	0.0	134.7	27.37
		2-21		P.p.	1.3294	1.2833	1.2841	0.1%	3.5183	0.0000	1.2726	1.2690	0.3%	1%		2.9663	1.2652	1.2650	2.4928	0.9002	0.9129	0.0	176.9	28.94
		2-22		P.p.	1.3234	1.3011	1.2922	0.7%	3.5105	0.0000	1.2744	1.2720	0.2%	1%		2.9599	1.2684	1.2691	2.1819	1.0401	0.9998	0.0	109.8	29.36
		2-23		P.p.	1.3452	1.3087	1.3164	0.6%	3.4539	0.0000	1.2977	1.2958	0.1%	1%		2.9329	1.2928	1.2922	2.3098	0.9006	0.9801	0.1	156.5	19.85
		2-24		P.p.	1.2476	1.2172	1.2152	0.2%	3.4490	0.0000	1.2045	1.2021	0.2%	1%		2.8474	1.1991	1.1991	2.1995	0.9014	0.9005	0.0	144.0	25.16

Treatment (trt)	wood species	Sample ID	Poplar's % of sapwood	Fungi exposed	Room Temperature Mass before trt (g)	Oven-dried mass before trt (m1)-- 1st trial	Oven-dried mass before trt (m1)-- 2nd trial	% of difference from 1st & 2nd Trial	Wet mass after trt (m2)	Retentions (kg/m3)	Oven-dried mass after trt (m3)--------- 1st trial	Oven-dried mass after trt (m3)--------- 2nd trial	% of difference from 1st & 2nd Trial	Mass gain %		Wet mass after leacheing	Oven-dried mass after Leaching---- 1st trial	Oven-dried mass after Leaching---- 2nd trial	Wet mass after fungi exposure (m4)	Oven-dried mass after fungi exposure (m5)---------- 1st trial	Oven-dried mass after fungi exposure (m5)---------- 2nd trial	% of difference from 1st & 2nd Trial	MC after decaying %	Mass loss %
1%	Hardwood	3-33		T.v.	1.3662	1.3132	1.3081	0.4%	3.7411	8.8666	1.3354	1.3338	0.1%	2%	non-leaching				2.7046	1.3299	1.3288	0.1	103.4	0.41
		3-34		T.v.	1.3982	1.3512	1.3428	0.6%	3.7732	8.8571	1.3664	1.3645	0.1%	2%					2.9693	1.3708	1.3701	0.1	116.6	-0.32
		3-37	80	T.v.	1.3874	1.3543	1.3500	0.3%	3.7188	8.6327	1.3577	1.3553	0.2%	1%					2.8054	1.3574	1.3561	0.1	106.7	0.02
		3-38		T.v.	1.5109	1.4712	1.4662	0.3%	3.8982	8.8630	1.4734	1.4669	0.4%	0%					2.9660	1.4346	1.4349	0.0	106.7	2.63
		3-39		T.v.	1.4066	1.3672	1.3645	0.2%	3.7877	8.8309	1.3754	1.3733	0.2%	1%					2.9186	1.3916	1.3908	0.1	109.7	-1.18
		3-40		T.v.	1.4135	1.3619	1.3651	0.2%	3.8513	9.0605	1.3755	1.3735	0.1%	1%					2.8024	1.3748	1.3755	0.1	103.8	0.05
		3-41		T.v.	1.3729	1.3223	1.3295	0.5%	3.7154	8.6950	1.3398	1.3376	0.2%	1%	leaching	3.4392	1.3211	1.3210	1.8060	1.1047	1.1136	0.0	63.5	17.55
		3-42		T.v.	1.3459	1.3051	1.3073	0.2%	3.6816	8.6527	1.3179	1.3080	0.8%	1%		3.3907	1.2985	1.2991	2.3156	1.0092	1.1078	0.1	129.4	23.42
		3-43		T.v.	1.3905	1.3581	1.3521	0.4%	3.8471	9.0926	1.3624	1.3568	0.4%	1%		3.4323	1.3424	1.3426	2.3495	0.9053	0.9041	0.0	159.5	33.55
		3-46		T.v.	1.3743	1.3339	1.3358	0.1%	3.7398	8.7609	1.3450	1.3425	0.2%	1%		3.3108	1.3240	1.3242	2.1556	0.8598	0.8584	0.0	150.7	36.07
		3-47		T.v.	1.4446	1.4022	1.4018	0.0%	3.8352	8.8681	1.4113	1.4088	0.2%	1%		3.4652	1.3909	1.3907	2.1506	0.9048	0.8983	0.0	137.7	35.89
		3-50	10	T.v.	1.3467	1.3148	1.3057	0.7%	3.6838	8.6665	1.3188	1.3158	0.2%	1%		3.3956	1.2981	1.2978	1.9026	1.0082	1.0591	0.1	88.7	23.55
		3-51		I.I.	1.3811	1.3462	1.3373	0.7%	3.7110	8.6505	1.3463	1.3440	0.2%	1%	non-leaching				2.8117	1.3744	1.3722	0.2	104.6	-2.09
		3-52	20	I.I.	1.2854	1.2472	1.2433	0.3%	3.5506	8.4085	1.2577	1.2554	0.2%	1%					3.1332	1.2968	1.2947	0.2	141.6	-3.11
		3-53	60	I.I.	1.3849	1.3472	1.3418	0.4%	3.7685	8.8437	1.3543	1.3516	0.2%	1%					2.9685	1.3689	1.3636	0.4	116.9	-1.08
		3-54		I.I.	1.4222	1.3788	1.3724	0.5%	3.8050	8.8652	1.3803	1.3775	0.2%	1%					2.7669	1.3907	1.3918	0.1	99.0	-0.75
		3-55		I.I.	1.4388	1.3920	1.3951	0.2%	3.8597	8.9818	1.4043	1.4022	0.1%	1%					2.6732	1.4019	1.4044	0.2	90.7	0.17
		3-57		I.I.	1.3747	1.3472	1.3365	0.8%	3.7189	8.6822	1.3450	1.3425	0.2%	1%					2.4779	1.3420	1.3401	0.1	84.6	0.22
		3-59	20	I.I.	1.2779	1.2469	1.2446	0.2%	3.4914	8.1880	1.2566	1.2538	0.2%	1%	leaching	3.2631	1.2369	1.2361	1.8458	1.2440	1.2435	0.0	48.4	1.00
		3-60	10	I.I.	1.3034	1.2692	1.2681	0.1%	3.5898	8.4610	1.2810	1.2779	0.2%	1%		3.3195	1.2607	1.2597	1.8749	1.2705	1.2612	0.0	47.6	0.82
		3-61	80	I.I.	1.3665	1.3382	1.3276	0.8%	3.6619	8.5069	1.3368	1.3338	0.2%	1%		3.3556	1.3172	1.3161	2.0654	1.3267	1.3234	0.0	55.7	0.76
		4-1		I.I.	1.3775	1.3317	1.3355	0.3%	3.7576	8.8269	1.3426	1.3405	0.2%	1%		3.2624	1.3217	1.3207	2.0035	1.3298	1.3208	0.0	50.7	0.95
		4-2		I.I.	1.3716	1.3352	1.3323	0.2%	3.7968	8.9814	1.3366	1.3344	0.2%	0%		3.2959	1.3166	1.3167	2.1722	1.3018	1.3034	0.0	66.9	2.60
		4-3		I.I.	1.3884	1.3582	1.3504	0.6%	3.7637	8.7948	1.3555	1.3527	0.2%	0%		3.2993	1.3373	1.3374	2.2233	1.3414	1.3405	0.0	65.7	1.04
	Softwood	2-37		P.p.	1.2850	1.2570	1.2508	0.5%	3.3576	7.6778	1.2588	1.2572	0.1%	1%	Non-leaching				3.2399	1.2637	1.2612	0.2	156.4	-0.39
		2-38		P.p.	1.2202	1.1809	1.1877	0.6%	3.2919	7.6684	1.1972	1.1953	0.2%	1%					3.0880	1.2018	1.2001	0.1	156.9	-0.38
		2-39		P.p.	1.3536	1.3167	1.3143	0.2%	3.4010	7.6046	1.3209	1.3189	0.2%	0%					2.9611	1.3263	1.322	0.3	123.3	-0.41
		2-40		P.p.	1.1996	1.1683	1.1580	0.9%	3.2462	7.6101	1.1670	1.1656	0.1%	1%					3.2344	1.1749	1.1714	0.3	175.3	-0.68
		2-41		P.p.	1.3827	1.3390	1.3303	0.6%	3.3784	7.4639	1.3369	1.3352	0.1%	0%					3.2330	1.3437	1.3413	0.2	140.6	-0.51
		2-42		P.p.	1.2125	1.1781	1.1748	0.3%	3.3868	8.0612	1.1839	1.1824	0.1%	1%					3.3553	1.1896	1.1876	0.2	182.1	-0.48
		2-43		P.p.	1.4838	1.4363	1.4424	0.4%	3.5104	7.5364	1.4492	1.4471	0.1%	0%	leaching	3.1809	1.4304	1.4301	2.3622	1.3850	1.3826	0.0	70.6	4.43
		2-44		P.p.	1.4033	1.3676	1.3664	0.1%	3.3916	7.3805	1.3736	1.3715	0.2%	1%		3.0409	1.3539	1.3543	2.1729	1.3060	1.3034	0.0	66.4	4.92
		2-45		P.p.	1.4147	1.3733	1.3798	0.5%	3.3565	7.2037	1.3851	1.3840	0.1%	0%		3.0405	1.3673	1.3667	2.8203	1.2934	1.1495	0.1	118.1	6.62
		2-46		P.p.	1.5326	1.4922	1.4964	0.3%	3.6040	7.6808	1.5029	1.5015	0.1%	0%		3.2735	1.4844	1.4843	3.1950	1.3843	1.3602	0.0	130.8	7.89
		2-47		P.p.	1.4190	1.3963	1.3834	0.9%	3.4303	7.4595	1.3898	1.3888	0.1%	0%		3.0747	1.3727	1.3728	1.7677	1.2893	1.1814	0.1	37.1	7.23
		2-48		P.p.	1.4017	1.3670	1.3661	0.1%	3.3453	7.2128	1.3735	1.3723	0.1%	1%		3.0456	1.3565	1.3559	2.3590	1.3356	1.3321	0.0	76.6	2.76

Treatment (trt)	wood species	Sample ID	Poplar's % of sapwood	Fungi exposed	Room Temperature Mass before trt (g)	Oven-dried mass before trt (m1)-- 1st trial	Oven-dried mass before trt (m1)-- 2nd trial	% of difference from 1st & 2nd Trial	Wet mass after trt (m2)	Retentions (kg/m3)	Oven-dried mass after trt (m3)--------- 1st trial	Oven-dried mass after trt (m3)--------- 2nd trial	% of difference from 1st & 2nd Trial	Mass gain %		Wet mass after leacheing	Oven-dried mass after Leaching---- 1st trial	Oven-dried mass after Leaching---- 2nd trial	Wet mass after fungi exposure (m4)	Oven-dried mass after fungi exposure (m5)---------- 1st trial	Oven-dried mass after fungi exposure (m5)---------- 2nd trial	% of difference from 1st & 2nd Trial	MC after decaying %	Mass loss %
10%	Hardwood	4-4	90	T.v.	1.383	1.3519	1.3434	0.6%	3.4079	75.2369	1.5046	1.5024	0.1%	11%	non-leaching				3.3730	1.3757	1.3727	0.2	145.2	8.57
		4-5		T.v.	1.3861	1.3483	1.3394	0.7%	3.7930	89.4169	1.5282	1.5259	0.2%	12%					3.5263	1.3796	1.3766	0.2	155.6	9.72
		4-6	85	T.v.	1.3842	1.3492	1.3477	0.1%	3.4910	78.1086	1.5158	1.5136	0.1%	11%					3.6189	1.3720	1.3786	0.5	163.8	9.49
		4-9		T.v.	1.3624	1.3251	1.3266	0.1%	3.7423	88.0357	1.5194	1.5173	0.1%	13%					3.8318	1.3641	1.3711	0.5	180.9	10.22
		4-11		T.v.	1.3757	1.3495	1.3413	0.6%	3.8516	91.4832	1.5456	1.5431	0.2%	13%					3.8994	1.3698	1.3664	0.2	184.7	11.37
		4-13		T.v.	1.4216	1.3873	1.3871	0.0%	3.6471	82.3615	1.5675	1.5648	0.2%	12%					3.8149	1.4145	1.4115	0.2	169.7	9.76
		4-14		T.v.	1.3814	1.3448	1.3468	0.1%	3.8247	90.3025	1.5466	1.5431	0.2%	13%	leaching	3.5914	1.3396	1.3381	2.4583	1.2513	1.2469	0.0	96.5	19.09
		4-15		T.v.	1.3578	1.3301	1.3243	0.4%	3.5939	82.7114	1.5045	1.5021	0.2%	12%		3.5171	1.3211	1.3190	2.3815	1.2556	1.2427	0.0	89.7	16.54
		4-16		T.v.	1.3529	1.3252	1.3181	0.5%	3.5633	81.8222	1.4973	1.4955	0.1%	12%		3.5176	1.3134	1.3118	2.2335	1.2973	1.284	0.0	72.2	13.36
		4-17	60	T.v.	1.372	1.3300	1.3343	0.3%	3.4141	75.7945	1.4958	1.4937	0.1%	11%		3.5152	1.3273	1.3258	2.2858	1.2957	1.2909	0.0	76.4	13.38
		4-18		T.v.	1.3698	1.3372	1.3301	0.5%	3.8061	90.2332	1.5316	1.5292	0.2%	13%		3.5625	1.3223	1.3208	2.4658	1.2197	1.1939	0.0	102.2	20.36
		4-21		T.v.	1.3925	1.3435	1.3440	0.0%	3.8603	91.7019	1.5489	1.5469	0.1%	13%		3.5112	1.3409	1.3396	2.2837	1.2608	1.2567	0.0	81.1	18.60
		4-23		I.I.	1.3836	1.3494	1.3421	0.5%	3.7646	88.2835	1.5371	1.5347	0.2%	13%	non-leaching				3.7911	1.3736	1.3713	0.2	176.0	10.64
		4-24		I.I.	1.3534	1.3133	1.3163	0.2%	3.7508	88.7208	1.5166	1.5137	0.2%	13%					3.6594	1.3544	1.3511	0.2	170.2	10.69
		4-25		I.I.	1.3886	1.3578	1.3510	0.5%	3.8266	90.2187	1.5515	1.5491	0.2%	13%					3.4686	1.3961	1.3921	0.3	148.4	10.02
		4-26		I.I.	1.3645	1.3352	1.3295	0.4%	3.6567	84.8105	1.5192	1.5184	0.1%	12%					3.3776	1.3690	1.3654	0.3	146.7	9.89
		4-27		I.I.	1.379	1.3663	1.3565	0.7%	3.9224	93.5095	1.5666	1.5647	0.1%	13%					3.6054	1.4002	1.4067	0.5	157.5	10.62
		4-28	70	I.I.	1.3777	1.3557	1.3551	0.0%	3.4818	77.5036	1.5173	1.5153	0.1%	11%					3.2645	1.3768	1.3735	0.2	137.1	9.26
		4-29	70	I.I.	1.3431	1.3001	1.3094	0.7%	3.4194	76.8950	1.4753	1.4733	0.1%	11%	leaching	3.4563	1.3049	1.3039	1.8098	1.3088	1.3035	0.0	38.3	11.29
		4-30		I.I.	1.3752	1.3373	1.3363	0.1%	3.7615	88.3819	1.5344	1.5329	0.1%	13%		3.5332	1.3302	1.3290	1.9167	1.3558	1.3512	0.0	41.4	11.64
		4-31		I.I.	1.3922	1.3493	1.3466	0.2%	3.8334	90.6268	1.5462	1.5450	0.1%	13%		3.5568	1.3445	1.3437	2.2458	1.3791	1.3612	0.0	62.8	10.81
		4-32		I.I.	1.3988	1.3518	1.3526	0.1%	3.8576	91.2901	1.5581	1.5570	0.1%	13%		3.6420	1.3460	1.3446	2.3124	1.2871	1.3094	0.0	79.7	17.39
		4-43		I.I.	1.3875	1.3582	1.3524	0.4%	3.8024	89.2857	1.5398	1.5385	0.1%	12%		3.6060	1.3405	1.3395	2.5356	1.3435	1.3184	0.0	88.7	12.75
		4-44		I.I.	1.4216	1.3942	1.3838	0.7%	3.8431	89.6246	1.5896	1.5888	0.1%	13%		3.6198	1.3815	1.3803	2.5990	1.3658	1.3419	0.0	90.3	14.08
	Softwood	2-61		P.p.	1.3863	1.3521	1.3416	0.8%	3.4380	76.3994	1.5211	1.5195	0.1%	12%	Non-leaching				3.4735	1.3823	1.3864	0.3	151.3	9.12
		2-62		P.p.	1.3233	1.2860	1.2830	0.2%	3.4934	80.5539	1.4775	1.4753	0.1%	13%					3.5288	1.3373	1.3316	0.4	163.9	9.49
		2-63		P.p.	1.2110	1.2018	1.1981	0.3%	3.4271	81.2318	1.3798	1.3777	0.2%	13%					3.4892	1.2334	1.2385	0.4	182.9	10.61
		2-64		P.p.	1.4676	1.4379	1.4297	0.6%	3.6429	80.6560	1.6223	1.6198	0.2%	12%					3.6932	1.4779	1.4718	0.4	149.9	8.90
		2-65		P.p.	1.5574	1.5190	1.5161	0.2%	3.7972	83.1305	1.7152	1.7123	0.2%	12%					3.8644	1.5798	1.5735	0.4	144.6	7.89
		2-66		P.p.	1.5092	1.4792	1.4690	0.7%	3.6975	81.2136	1.6627	1.6607	0.1%	12%					3.7479	1.5342	1.5379	0.2	144.3	7.73
		2-67		P.p.	1.3568	1.3222	1.3208	0.1%	3.4529	77.7004	1.5067	1.5044	0.2%	12%	leaching	3.1096	1.3141	1.3129	2.9662	1.2892	1.2639	0.0	130.1	14.44
		2-68		P.p.	1.4302	1.4033	1.3915	0.8%	3.5450	78.4803	1.5808	1.5776	0.2%	12%		3.2262	1.3858	1.3833	2.7471	1.3120	1.2867	0.0	109.4	17.00
		2-69		P.p.	1.2466	1.2010	1.2079	0.6%	3.4764	82.6713	1.4105	1.4079	0.2%	14%		3.0777	1.2028	1.2010	2.2190	1.2090	1.184	0.0	83.5	14.29
		2-70		P.p.	1.4208	1.3693	1.3666	0.2%	3.4040	74.2493	1.5406	1.5377	0.2%	11%		3.0213	1.3599	1.3574	2.1623	1.3281	1.3223	0.0	62.8	13.79
		2-71		P.p.	1.2530	1.2082	1.2064	0.1%	3.5672	86.0350	1.4205	1.4179	0.2%	15%		3.0584	1.2015	1.2000	2.4067	1.2391	1.2146	0.0	94.2	12.77
		2-72		P.p.	1.2403	1.2066	1.1984	0.7%	3.5527	85.7981	1.4115	1.4088	0.2%	15%		2.9882	1.1918	1.1909	1.8958	1.2254	1.2298	0.0	54.7	13.18

Treatment (trt)	wood species	Sample ID	Poplar's % of sapwood	Fungi exposed	Room Temperature Mass before trt (g)	Oven-dried mass before trt (m1)-- 1st trial	Oven-dried mass before trt (m1)-- 2nd trial	% of difference from 1st & 2nd Trial	Wet mass after trt (m2)	Retentions (kg/m3)	Oven-dried mass after trt (m3)--------- 1st trial	Oven-dried mass after trt (m3)--------- 2nd trial	% of difference from 1st & 2nd Trial	Mass gain %		Wet mass after leacheing	Oven-dried mass after Leaching---- 1st trial	Oven-dried mass after Leaching---- 2nd trial	Wet mass after fungi exposure (m4)	Oven-dried mass after fungi exposure (m5)---------- 1st trial	Oven-dried mass after fungi exposure (m5)---------- 2nd trial	% of difference from 1st & 2nd Trial	MC after decaying %	Mass loss %
15%	Hardwood	4-35		T.v.	1.3616	1.3244	1.3239	0.0%	3.1711	100.9767	1.6280	1.6255	0.2%	19%	non-leaching				3.7374	1.4014	1.4074	0.4	166.7	13.92
		4-36		T.v.	1.3861	1.3597	1.3508	0.7%	3.2023	101.2117	1.6544	1.6518	0.2%	18%					3.7889	1.4287	1.4253	0.2	165.2	13.64
		4-37		T.v.	1.3388	1.3239	1.3160	0.6%	3.2168	103.9067	1.6113	1.6076	0.2%	18%					3.7673	1.3892	1.3857	0.3	171.2	13.78
		4-38		T.v.	1.4094	1.3771	1.3753	0.1%	3.1781	98.5496	1.6275	1.6237	0.2%	15%					3.6544	1.4438	1.4403	0.2	153.1	11.29
		4-39	80	T.v.	1.3724	1.3383	1.3386	0.0%	3.1572	99.4133	1.5808	1.5779	0.2%	15%					3.6076	1.4109	1.4121	0.1	155.7	10.75
		4-40	80	T.v.	1.3538	1.3219	1.3162	0.4%	3.1065	97.8663	1.5528	1.5503	0.2%	15%					3.6336	1.3806	1.3823	0.1	163.2	11.09
		4-41	80	T.v.	1.3631	1.3285	1.3177	0.8%	3.1501	100.1682	1.5571	1.5541	0.2%	15%	leaching	3.6481	1.3209	1.3198	2.6450	1.3645	1.3408	0.0	93.8	12.37
		4-42		T.v.	1.3916	1.3493	1.3460	0.2%	3.3478	109.4278	1.6414	1.6388	0.2%	18%		3.6751	1.3417	1.3402	2.2301	1.3904	1.3659	0.0	60.4	15.29
		4-43		T.v.	1.3379	1.2901	1.2935	0.3%	3.2310	105.9129	1.5784	1.5756	0.2%	18%		3.5771	1.2919	1.2906	2.4552	1.3429	1.3178	0.0	82.8	14.92
		4-45		T.v.	1.3335	1.3092	1.3038	0.4%	3.2361	105.6286	1.5929	1.5895	0.2%	18%		3.5678	1.3005	1.2984	2.2989	1.3470	1.3223	0.0	70.7	15.44
		4-47		T.v.	1.3721	1.3421	1.3345	0.6%	3.2583	105.1640	1.6356	1.6324	0.2%	18%		3.6425	1.3313	1.3306	2.3007	1.2723	1.2476	0.0	80.8	22.21
		4-50		T.v.	1.3556	1.3383	1.3383	0.0%	3.2285	103.3273	1.5963	1.5934	0.2%	16%		3.5407	1.3172	1.3146	2.3004	1.2736	1.2498	0.0	80.6	20.22
		4-51	30	I.I.	1.3711	1.3469	1.3537	0.5%	3.0191	91.0386	1.5709	1.5682	0.2%	14%	non-leaching				3.4216	1.4065	1.4086	0.1	143.3	10.47
		4-52		I.I.	1.3622	1.3306	1.3242	0.5%	3.2554	105.5685	1.6275	1.6234	0.3%	19%					3.6665	1.4099	1.4024	0.5	160.1	13.37
		4-53		I.I.	1.4323	1.3803	1.3893	0.7%	3.3269	105.9184	1.6911	1.6879	0.2%	18%					3.6515	1.4748	1.4717	0.2	147.6	12.79
		4-55		I.I.	1.3856	1.3478	1.3403	0.6%	3.2417	103.9395	1.6203	1.6177	0.2%	17%					3.4377	1.4238	1.4228	0.1	141.4	12.13
		4-56		I.I.	1.3391	1.2959	1.2910	0.4%	3.1938	104.0160	1.5766	1.5736	0.2%	18%					3.5550	1.3678	1.3656	0.2	159.9	13.24
		4-57		I.I.	1.4001	1.3591	1.3556	0.3%	3.1425	97.6804	1.6187	1.6150	0.2%	16%					3.5257	1.4267	1.4281	0.1	147.1	11.86
		4-58		I.I.	1.3655	1.3472	1.3355	0.9%	3.2619	105.3061	1.6134	1.6107	0.2%	17%	leaching	3.6246	1.3314	1.3279	2.4557	1.3690	1.344	0.0	79.4	15.15
		4-59	85	I.I.	1.394	1.3431	1.3499	0.5%	3.3022	106.7219	1.5932	1.5902	0.2%	15%		3.6450	1.3503	1.3472	2.5148	1.3474	1.3407	0.0	86.6	15.43
		4-60		I.I.	1.3863	1.3583	1.3532	0.4%	3.2946	106.1261	1.6613	1.6588	0.2%	19%		3.6468	1.3418	1.3399	2.3907	1.3651	1.3626	0.0	75.1	17.83
		4-61		I.I.	1.4141	1.3792	1.3696	0.7%	3.2025	100.1950	1.6741	1.6711	0.2%	18%		3.7022	1.3654	1.3645	2.4964	1.3614	1.337	0.0	83.4	18.68
		4-62		I.I.	1.3981	1.3581	1.3494	0.6%	3.1757	99.8342	1.5897	1.5873	0.2%	15%		3.5762	1.3461	1.3452	2.5510	1.3248	1.3098	0.0	92.6	16.66
		4-63		I.I.	1.3918	1.3477	1.3384	0.7%	3.1777	100.5448	1.5960	1.5932	0.2%	16%		3.5671	1.3357	1.3346	2.4998	1.3079	1.2813	0.0	91.1	18.05
	Softwood	2-85		P.p.	1.2332	1.1915	1.1963	0.4%	3.0152	99.4297	1.4894	1.4874	0.1%	20%	Non-leaching				3.2735	1.2698	1.2659	0.3	157.8	14.74
		2-86		P.p.	1.5053	1.4682	1.4609	0.5%	3.2764	99.2438	1.7481	1.7454	0.2%	16%					3.5402	1.5404	1.5449	0.3	129.8	11.88
		2-87		P.p.	1.4512	1.4183	1.4069	0.8%	3.1784	96.8386	1.6861	1.6828	0.2%	17%					3.6577	1.5004	1.5037	0.2	143.8	11.01
		2-88		P.p.	1.1972	1.1519	1.1592	0.6%	3.0023	100.7526	1.4650	1.4622	0.2%	21%					3.5451	1.2604	1.2645	0.3	181.3	13.97
		2-89		P.p.	1.2926	1.2434	1.2466	0.3%	3.0919	100.8728	1.5469	1.5439	0.2%	19%					3.2638	1.3466	1.3425	0.3	142.4	12.95
		2-90		P.p.	1.2087	1.1700	1.1630	0.6%	3.0017	100.5120	1.4667	1.4640	0.2%	21%					3.1925	1.2715	1.2758	0.3	151.1	13.31
		2-91		P.p.	1.3873	1.3330	1.3314	0.1%	3.0550	94.2201	1.6065	1.6040	0.2%	17%	leaching	3.1874	1.3278	1.3255	2.6078	1.3801	1.3558	0.0	89.0	14.09
		2-92		P.p.	1.5626	1.4991	1.5015	0.2%	3.3489	100.9876	1.7918	1.7885	0.2%	16%		3.5099	1.4983	1.4964	3.4609	1.5200	1.5259	0.0	127.7	15.17
		2-93		P.p.	1.2142	1.1706	1.1667	0.3%	3.0127	100.9111	1.4638	1.4609	0.2%	20%		3.1581	1.1655	1.1641	2.4123	1.2099	1.1861	0.0	99.4	17.35
		2-94		P.p.	1.5002	1.4303	1.4382	0.6%	3.2346	98.1997	1.7268	1.7241	0.2%	17%		3.3957	1.4405	1.4392	2.7756	1.4758	1.4515	0.0	88.1	14.54
		2-95		P.p.	1.4257	1.3732	1.3797	0.5%	3.1242	95.3626	1.6511	1.6489	0.1%	16%		3.2957	1.3703	1.3687	2.9013	1.4121	1.3872	0.0	105.5	14.48
		2-96		P.p.	1.2128	1.1761	1.1694	0.6%	3.0102	100.6268	1.4699	1.4672	0.2%	20%		3.1382	1.1679	1.1664	2.4370	1.2023	1.179	0.0	102.7	18.21

Appendix 9: Significant letters

Retention

Sample_no	Estimate mean	Significant letter (Retention)													
sample no 20	104.94	A													
sample no 22	103.12	A													
sample no 21	101.36	A	B	C	D										
sample no 19	100.32	A	B	C	D										
sample no 23	99.6067	A	B	C	D										
sample no 24	98.385	A	B	C	D										
sample no 16	87.685		B	C	D	E	F	G							
sample no 15	87.1733			C	D	E	F	G							
sample no 14	85.425				D	E	F	G							
sample no 13	84.1083					E	F	G							
sample no 18	80.8217						F	G							
sample no 17	80.53							G							
sample no 7	8.8517								H	I	J	K	L		
sample no 8	8.7883									I	J	K	L		
sample no 9	8.7383										J	K	L		
sample no 10	8.6267											K	L		
sample no 11	7.68												L	M	
sample no 12	7.4117													M	

Mass Gain

Sample No.	Estimate mean	Significant letter (Mass Gain)																	
smaple no 23	18.9434	A																	
smaple no 24	17.8694	A	B																
smaple no 20	17.357	A	B																
smaple no 21	16.9931	A	B																
smaple no 19	16.902	A	B																
smaple no 22	16.7478	A	B																
smaple no 18	13.3568		B	C	D	E	F	G											
smaple no 15	12.5674			C	D	E	F	G											
smaple no 16	12.5619				D	E	F	G											
smaple no 14	12.341					E	F	G											
smaple no 17	12.2105						F	G											
smaple no 13	11.9291							G											
smaple no 6	1.109								H	I	J	K	L	M	N	O	P	Q	R	
smaple no 7	1.0627									I	J	K	L	M	N	O	P	Q	R	S
smaple no 5	0.8849										J	K	L	M	N	O	P	Q	R	S
smaple no 1	0.8803											K	L	M	N	O	P	Q	R	S
smaple no 3	0.8645												L	M	N	O	P	Q	R	S
smaple no 2	0.8267													M	N	O	P	Q	R	S
smaple no 8	0.7799														N	O	P	Q	R	S
smaple no 9	0.766															O	P	Q	R	S
smaple no 4	0.6835																P	Q	R	S
smaple no 11	0.6604																	Q	R	S
smaple no 10	0.6462																		R	S
smaple no 12	0.468																			S

Mass Loss

Sample no.	Estimate mean	Significant letter (Mass Loss)																				
sample no 2	35.5005	A																				
sample no 8	27.2604	A	B	C	D																	
sample no 6	25.1562	A	B	C	D																	
sample no 3	20.4367		B	C	D																	
sample no 1	18.7859			C	D																	
sample no 5	16.4743				D	E	F	G	H	I												
sample no 4	14.2298					E	F	G	H	I												
sample no 23	12.9771						F	G	H	I												
sample no 19	12.4116							G	H	I	J	K										
sample no 21	12.3097								H	I	J	K										
sample no 15	10.1861									I	J	K										
sample no 13	9.8556										J	K	L									
sample no 17	8.9579											K	L									
sample no 14	4.8148												L	M	N	O	P	Q			U	
sample no 12	4.4127													M	N	O	P	Q			U	
sample no 18	0.5158														N	O	P	Q	R	S	T	U	V
sample no 7	0.2697															O	P	Q	R	S	T	U	V
sample no 16	0.08196																P	Q	R	S	T	U	V
sample no 22	-0.06487																	Q	R	S	T	U	V
sample no 10	-0.3113																		R	S	T	U	V
sample no 11	-0.4749																			S	T	U	V
sample no 9	-1.1056																				T	U	V
sample no 20	-1.1187																					U	V
sample no 24	-2.9405																						V