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Fungal Pigments: An Investigation into their Environmental Stability and Application to Conservation

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Fungal Pigments:

An Investigation into their Environmental Stability and Application to Conservation

A thesis submitted in partial satisfaction of the requirements for the degree

Master of Arts in Conservation of Cultural Heritage

by

Tamara Dissi

2023

ABSTRACT OF THE THESIS

Fungal Pigments:
An Investigation into their Environmental Stability and Application to Conservation

by

Tamara Dissi

Master of Arts in Conservation of Cultural Heritage
University of California, Los Angeles, 2023
Professor Ellen J. Pearlstein, Chair

Spalting is the result of fungal metabolic processes that create unique, colored patterns inside of wood. As such, spalted wood has been historically prized for its beauty. Fungal pigments not only beautify wood through the spalting process though, they also impart it with strong environmental resistance. This is owed to the fact that fungi secrete pigments in wood for use in capturing and defending resources necessary for survival against competing fungal species. Pigments also provide protective properties for fungi such as light and ultraviolet (UV) resistance, bacterial and insect resistance, and prevention of desiccation in extreme environmental conditions. For these reasons, there is growing interest in fungal pigments due to their light fast, color fast, and UV light stable properties. In addition to their extraordinary environmental stability, these naturally sourced pigments are also affordable and sustainable.

Spalted wood is very rare to find in the wild as the fungi require very specific environmental conditions for these processes to occur. In 2007, Dr. Seri Robinson spearhead research into spalting fungi. Robinson, a professor at Oregon State University and bio-artist, developed a process in which to extract pigments from three fungi species and use them to artificially induce spalting in the span of ten days, a process which previously took up to two years in the wild.

The extraordinary environmental stability of fungal pigments makes them a material that could have potentially significant applications to the field of conservation including new lightfast pigmented coatings or inpainting mediums. In the literature, the spalting fungal pigments are purported to be light and UV resistant, however, their color stability has not been specifically measured nor has it been evaluated to museum standards. To assess their degree of lightfastness, accelerating aging experiments on fungal pigmented paper and wood coupons were conducted and subsequently compared to blue wool standards to extrapolate qualitative measurements.

This full-spectrum light aging experiment established that fungal pigments are not more lightfast than many other natural dyes and colorants. To quantify the degree of color change, describe as ΔE^*, values were calculated from CIELab measurements from before and after accelerated light aging. The averaged ΔE^* values are as follows: blue pigment on paper, 8.98; blue pigment on wood, 11.70; red pigment on paper, 2.87; red pigment on wood, 6.94; purple pigment on paper, 8.98; purple pigment on wood, 14.88; yellow pigment on paper, 5.95; and yellow pigment on wood, 6.74. The results of this study revealed that fungal pigments are not significantly light stable according to museum standards. Given these results, fungal pigments in their raw form are not recommended for conservation applications, however, further study could look into their stability when incorporated into other materials.

The thesis of Tamara Dissi is approved.

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University of California, Los Angeles

2023

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1. Introduction

Certain fungi can create unique and beautifully colored patterns in wood through the secretion of pigments in a naturally occurring phenomenon that is referred to as spalting. Since this colorful transformation occurs within the wood matrix, finding spalted wood in the wild requires skill and a decent amount of luck. Spalting results from fungal metabolic processes that only occur under very specific environmental conditions so artificially inducing it with do-it-yourself recipes are largely unsuccessful. Due to its rarity, spalted wood has been artistically prized for centuries. Beginning in the 15th century, for example, spalted wood was sometimes incorporated into intarsia and marquetry which involved fitting small, cut pieces of blue-green colored wood into a solid wood background, the color of which is still retained to this day (Blanchette, et al., 1992; Robinson, et al., 2016) (fig. 1).

The use of spalted wood nearly disappeared by the turn of the industrial revolution with the introduction of more affordable synthetic colorants in the 19th century. Their low costs, ease of production, and superior coloring properties established the high demand and use of synthetic colorants in the market for over a decade (Venil, et. al., 2020). In more recent years, however, caution against the use of synthetic colorants has increased due to their adverse impacts to the environment and detrimental effects to human health. Some of the disadvantages of synthetic colorants such as poor biodegradation, high energy and water usage during production, and the potential to cause cancers and other health concerns have increased the demand for natural, organic, and eco-friendly pigments (Lagashetti, et al., 2019). In recent years, fungi have become a prominent source for natural and affordable eco-friendly pigments. Fungal pigments are currently being used in the food, pharmaceutical, cosmetic, and textile industries (Meruvu and Santos, 2021).

Figure 1: Wood with a green stain caused by this fungus was used as intarsia inlay depicting a bird and leaves in the 1587 pulpit in St. Mary's Church in Greifswald, Germany (top). The fungi in the genus Chlorociboria have bright blue-green fruiting bodies (bottom left) and cause blue-green pigmentation throughout the wood in which they grow (bottom right). Images from Robinson, 2017.

In addition to their low cost and sustainability, fungal pigments are of growing interest due to their purported extraordinary environmental stability. These pigments are allegedly lightfast, color fast, and UV light stable and studies have shown that they can reliably dye a number of substrates, including wood, bamboo, paper, and textiles (Gutierrez and Robinson, 2017; Hinsch, et al., 2015; Palomino Agurto, et al., 2020). Although lightfastness and color change weren't specifically measured in a study that explored the different techniques for applying fungal pigments on textiles, the authors did remark that the dyed textiles did not exhibit perceivable fading over a course of a week (Weber, 2014;). In another study, pigments from *S. cuboideum* had superior UV stability when compared to other biological pigments and had the

potential to remain vibrant under all light conditions. That study also found that when these pigments were incorporated into protective wood coatings, they performed better than coatings without pigments likely due to the ability of pigments to absorb UV energy (Robinson et al., 2013). In another study that assessed the resistance of pigments from C. *aeruginosa* and S. *cuboideum* to UV exposure, it was found that they were unable to protect the lignin in wood from photodegradation. However, FTIR peaks associated with these pigments decline less after UV exposure than the lignin peaks which suggests that the fungal pigments were more UV stable and resistant to degradation than the wood itself (Beck, et al., 2014).

Fascinatingly, in addition to being bio-available sources of color, some fungal pigments hold additional properties that are valuable in other industries. For example, the blue-green fungal pigment known as xylindein that is extracted from *C. aeruginosa* was found to be exceptionally heat and photostable, with film properties unaffected up to 180 °C and degradation occurring at temperatures as high as 210 °C. As a coating and exposed to a weathering chamber, xylindein also showed potential to protect wood from degradation (Harrison, et al., 2017). The opto-electronic properties and high electron mobility of xylindein shows promise for the development of sustainable and organic semiconductor materials (Geisbers, 2019).

The prized qualities of fungal pigments are being exploited to advance study and develop products in many different fields and disciplines. Should fungal pigments truly have extraordinary environmental stability, they could become invaluable inpainting or coating materials in the field of conservation, especially as we seek to incorporate more sustainable materials and practices. To date, however, the light stability of these pigments has not been specifically measured nor have they been evaluated to museum standards. This is problematic because the preservation of cultural heritage requires very specific environmental parameters.

What might be defined as ‘light stable’ in other fields may not translate to how conservation defines light stability.

To assess the degree of light stability of fungal pigments, this research aims to specifically test and measure the color change of four different fungal pigments through an accelerated light aging experiment. The pigments were sourced from the Applied Mycology Lab at the Oregon State University and include a blue-green pigment extracted from *Chlorociboria aeruginosa*, a red pigment extracted from *Scytalidium cuboideum*, and yellow and purple pigments both extracted from *Scytalidium ganodermophthorum*. The pigments were coated onto both paper and wood coupons and subsequently subjected to accelerated light aging. CIELab color values were measured before and after aging with a portable integrated sphere spectrophotometer to determine the delta in color change. Qualitative assessments were also achieved by comparing the degree of color fading to ISO Blue Wool Standards (BWS), an established method of assessing lightfastness in museum collections. Ideally, this research will be used to encourage further study into the extraordinary properties of fungi and the capacities to which they can provide sustainable solutions in conservation practice.

2. Literature Review

2.1 Light Degradation to Collection Materials and Museum Standards

Radiation from light, ultraviolet (UV), and infrared (IR) is understood to be one of the key agents of deterioration to collection materials (Michalski, 2007). Light, by definition, is the band of radiation between 400 nm and 700 nm in the electromagnetic spectrum to which the

human eye is sensitive. The higher energy wavelengths of UV, and lower wavelengths of IR, fall within the 200 nm to 400 nm and 700 nm to 1mm, respectively. Radiation is comprised of energetic photon ‘bullets’ that have the potential to induce irreversible degradation to materials by triggering a complex series of chemical changes referred to as photochemical damage (Conn, 2012). When this energy is absorbed by materials, it excites atoms causing them to become much more reactive and liable to change (Feller, 1964). This can lead to degradation by increasing the vibration of the molecules within materials, encouraging them to expand, cleave, and re-bond or cross-link. These molecular activities can cause materials to fade, discolor, yellow, darken, oxidize, become brittle, crack, weaken, or a combination of the above listed. (Conn, 2012).

One way to preserve collection materials is to limit light exposure. Visible light levels are measured in lux ("lumens per square meter") or footcandles. One footcandle equals about 11 lux. The total exposure or dose of light on a surface is the product of light intensity (lux) and time (hours). In museums, the practical unit is millions of lux hours, abbreviated Mlx h, and pronounced "mega lux hours." Photochemical damage to collection materials is a factor of both lighting intensity and duration which means that limited exposure to a high-intensity light will produce the same amount of damage as a long exposure to a low-intensity light. This led to the development of the reciprocity principle which implies that damage can be mitigated by controlling one or both of these factors (Horelick, et al., 2011). For example, an artifact displayed at 150 lux for 100 years will hypothetically fade at the same rate as an artifact displayed at 5000 lux for 3 years.

Since color change is a key indication of photochemical damage, it can be a useful property to gauge whether damage has been incurred by a material. An issue, however, arises in the fact that color is not a material property but rather a human perception (Ford and Korenburg,

2022). Color difference and lightfastness, therefore, depend on how they are measured. Additionally, materials have differing levels of light sensitivity depending on their composition. Accordingly, museums adopt qualitative and quantitative methods by which to evaluate this in a more standardized manner. Two such methods utilized in this research are the International Standardization Organization for Blue Wool Standards (ISO BWS) and the Commission Internationale de l´Eclairage L*a*b* (CIELab) color space.

ISO BWS is a general classification system for lightfastness of collection materials. These standards help estimate the damage that might result to an artifact from intensities of light and lengths of exposure. ISO BWS cards contain eight dyed wool swatches, numbered 1 through 8, which fade at different known rates under light exposure. Each swatch fades at a rate two times as fast as the next with BSW #8 being the most lightfast and BSW #1 being the most sensitive. Materials rated as ISO 1, 2, or 3 are defined as highly light sensitive; ISO 4, 5, and 6 are defined as having medium sensitivity; and ISO 7 and 8 are defined as having low sensitivity (CIE 2004). The abovementioned exposure of 150 lux for 100 years would cause significant fading of Blue Wool standard 4 and below (Michalski, 2007).

One method to utilize ISO BWS cards is by placing it alongside a material that is exposed to a light source. Both the material and the sample card are partly covered, and visual comparisons are made after a length of time to match the rate of fading of the material to that of one of the wool samples. Since the sensitivity of the first few samples on the card corresponds to light sensitive materials such as paper and textiles, the results give a general idea of the amount of damage that can be expected if materials were exhibited for the same period of time at the current light level in that location (Patkus, 2008).

To quantitively measure color of a material, reflective spectrophotometric measurements can be conducted. Differences in color can be calculated using the L*a*b* values from the CIElab color space, a color recording system commonly adapted in conservation studies that quantifies a color's properties and determines the numerical differences between shades. The CIELab color space was invented in 1976 to connect the numerical wavelengths from the electromagnetic spectrum with human color perception (Sharma, et al., 2005). It uses L*, a* and b* values as coordinates to plot colors in a three-dimensional sphere (fig. 2). The L* represents the lightness of a color on a scale from zero (black) to 100 (white). The a* axis is relative to the green–red opponent colors, with negative values toward green and positive values toward red. The b* axis represents the blue–yellow opponents, with negative numbers toward blue and positive toward yellow. Values for a* and b* that are close to zero will tend to appear grey. In this color space, numerical differences between values roughly correspond to the amount of change humans perceive between colors (Phillips 2023).

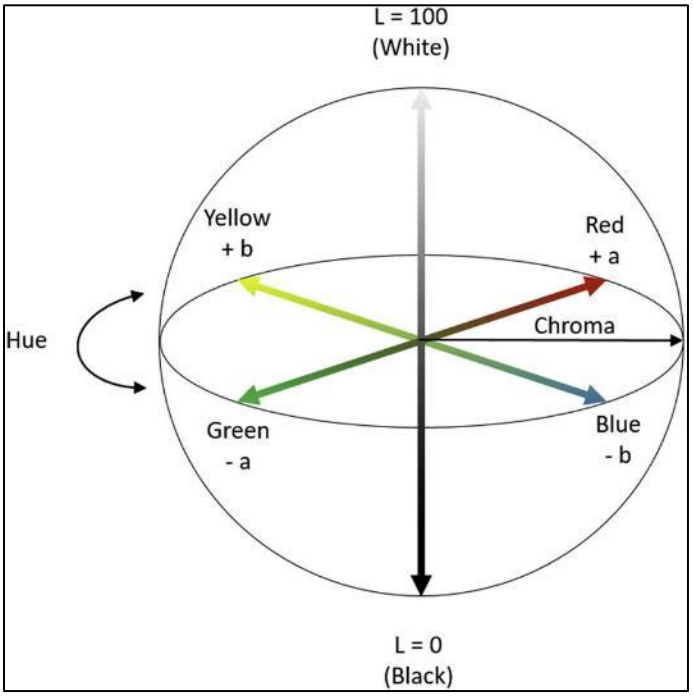


Figure 2: CIELab color space illustrating L, a*, and b* values. Image from Bao, 2020.*

2.2 Spalting Fungi Colonization and the Biomechanics of Wood Decay

To discuss spalting, familiarity with the anatomy and growth biomechanisms of both wood and fungi are first necessary. Wood is a porous and hygroscopic interconnected matrix of cells composed of cellulose, hemicellulose, and lignin (fig. 3). Cellulose and hemicellulose are polysaccharide biomolecules that serve as the structural components of the plant cell wall while lignin is an aromatic biomacromolecule that glues them together.

Hardwood trees grow concentrically around a central pith by forming a ring of new cells just within the bark. The inner space of plant cells is referred to as the lumen, and the permeable areas between cell walls are preferred to as pits. Like all plants, trees are heterotrophs and can synthesize their own food. Nutrients are transported vertically through vessels which are a stacked series of relatively large cells with open ends. In cross sections, the vessels appear as holes and are known as pores. Nutrients are stored and horizontally transported through ray cells.

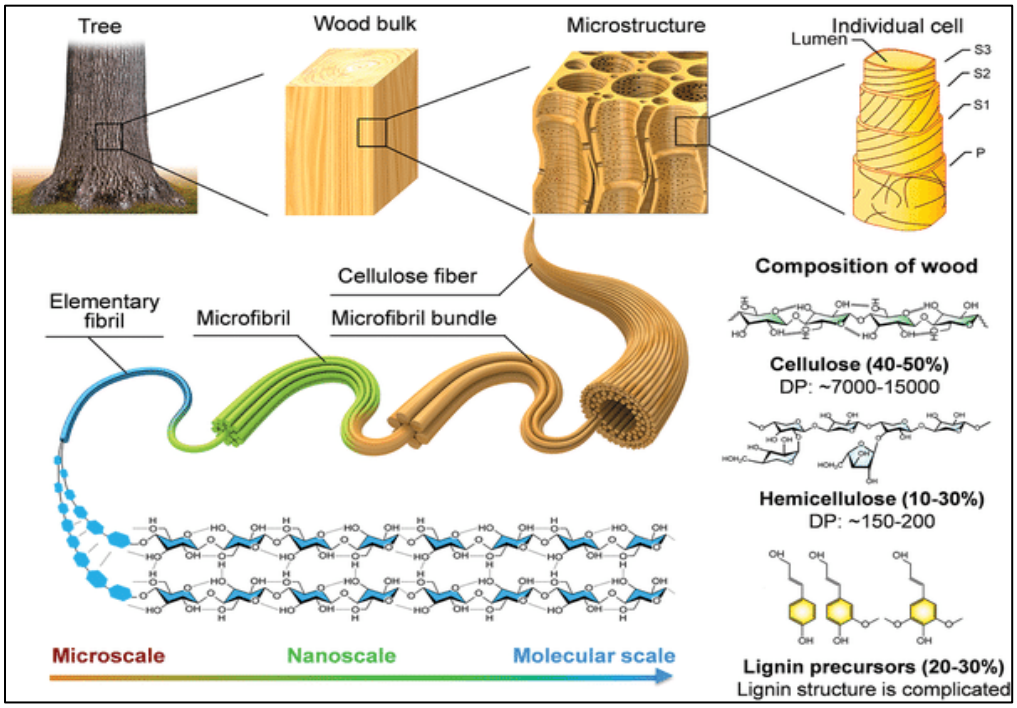


Figure 3: Wood's hierarchical structure and molecular compositions. Image from Chaoji and Hu, 2021.

Neither animal nor plant, fungi are spore-producing eukaryotic organisms comprising their own kingdom of life. Aside from yeast which are unicellular, fungi are generally multicellular organisms. The living body of the fungus, the mycelium, is usually imbedded within a substrate such as soil or wood and hidden from view (fig 4). The mycelium is composed of a radially expanding network of branching filaments called hyphae. Fungi reproduce by releasing spores from reproductive bodies commonly known as mushrooms which are usually the only part of the fungus that is visible. Like plants, fungal cells have walls, but they are mostly comprised of chitin which is the same material in the hard outer shells of insects and other arthropods. Unlike plants and animals, however, fungi cannot synthesize or ingest their food. Instead, fungi feed by absorbing nutrients from the organic material in which they live. Wood is an especially attractive food source for fungi due to their carbohydrate rich composition (Ahmadjian, et al., 2023).

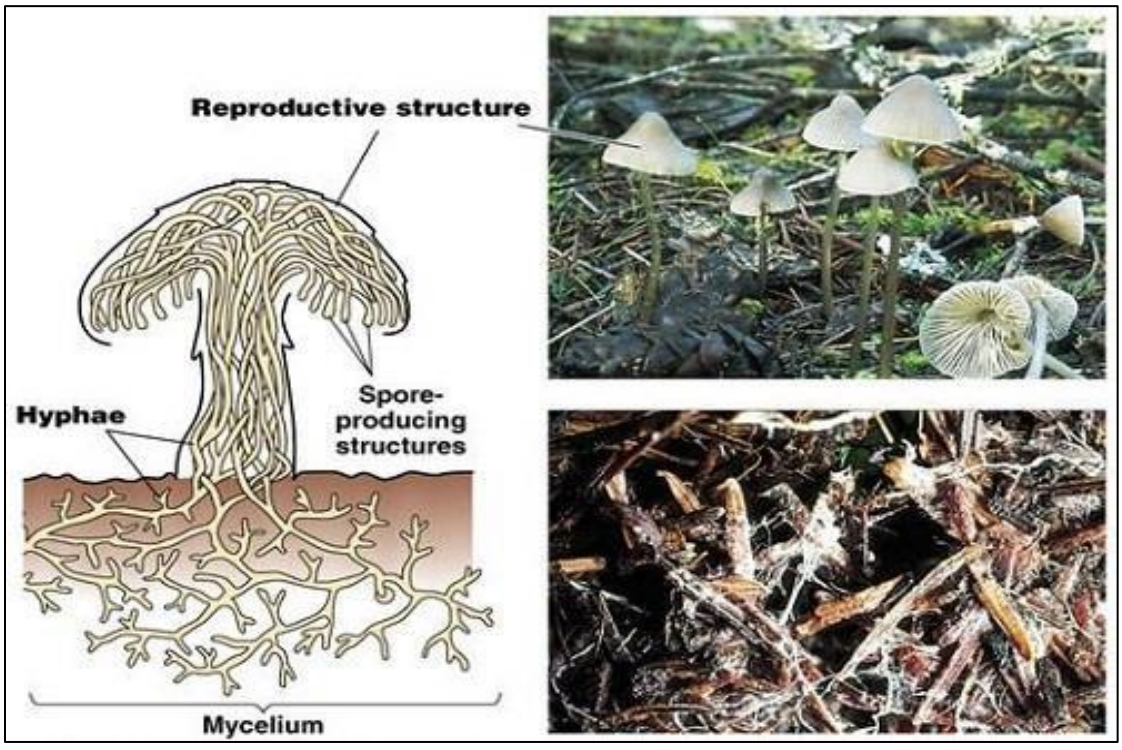


Figure 4: Fungal structure. The body of the fungi is called the mycelium which is composed of hyphae. Spores are released for reproduction through 'reproductive bodies' commonly known as mushrooms. Image from Francisco, 2017.

The fungi that are capable of spalting are caused by certain wood-inhabiting fungi within the Basidiomycota and Ascomycota phyla. They begin colonizing in the vessel and ray cells where resources are the easiest to procure due to the distribution of free sugars (Gutierrez, et al., 2018). This type of colonization changes the permeability of the wood but does not alter its strength. Once the easily available sugars have been utilized, only fungi with specific wood degrading enzymes or secondary metabolites such as pigments can continue colonization (Robinson, 2010). The unique patterns induced by spalting are therefore the results of biodegradative processes that alter the wood structure on both a visual and a cellular level. Bleaching, zoning, and pigmentation are the three characteristic patterns induced by spalting and their formation depends on the colonization strategy of a particular fungi and the biochemistry of the decay process (Morris, et al., 2021) (fig. 5).

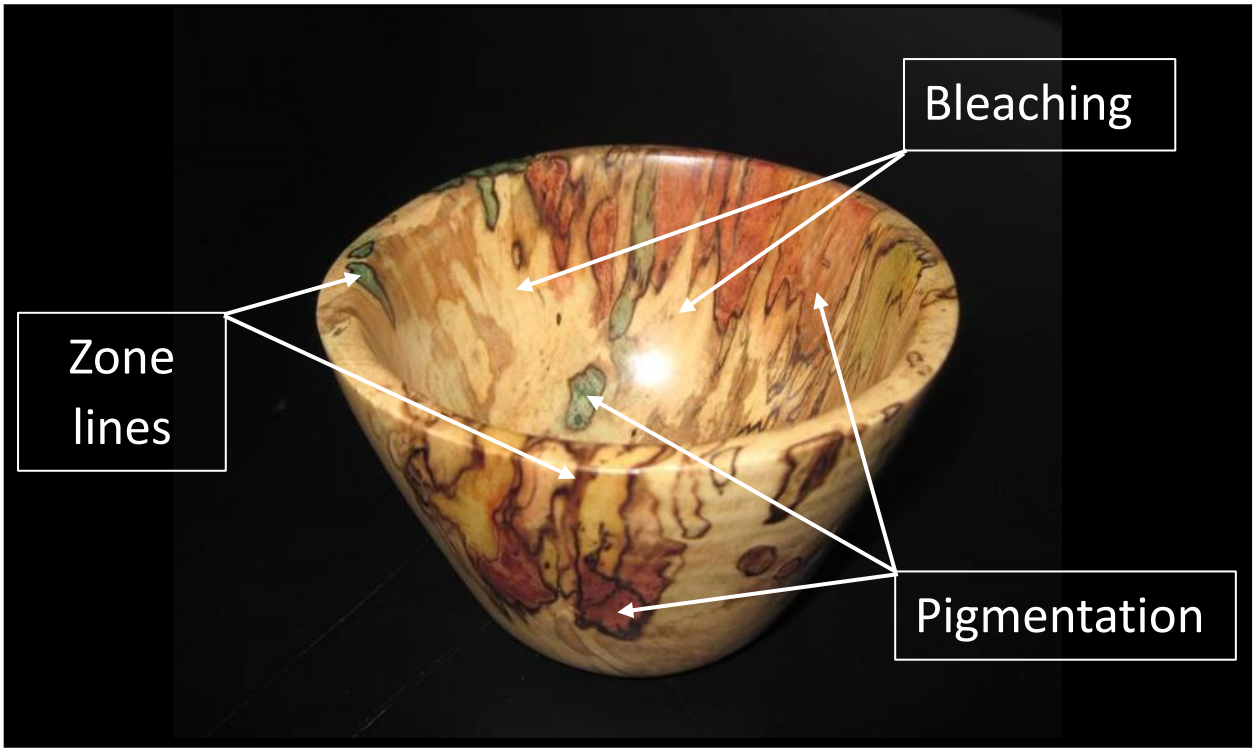


Figure 5: Three types of patterns exhibited in spalting wood including zone lines, bleaching, and pigmentation.

Basidiomycetes are known as wood-decay fungi because of their ability to rapidly metabolize cellulose and hemicellulose. This phylum is subdivided into brown-rot and white-rot fungi, with the latter having the additional unique ability to produce an enzyme called laccase that can break down phenolic compounds like lignin (Andlar, et al., 2018). By removing lignin and other colored wood components, white-rot basidiomycetes are responsible for the color loss, or bleaching, in spalted wood (Gutierrez, 2016). Lightning in color can also be due to a buildup of white mycelium in select areas (Van Court and Robinson, 2019). Since bleaching is caused by the degradation of cells walls, it also causes structural change to the wood's composition. Bleached wood is often weakened and porous due to the decrease in mass (Robinson, 2011).

Bleaching is often accompanied by zoning, or zone lines, which refers to the dark narrow winding patterns seen in spalted wood that delineate fungal territory. Zone lines can exhibit different colorations depending on their composition but are most commonly composed of melanin which generates colors raging from black to light brown (Robinson, 2016). White-rot basidiomycetes produce these demarcations as a defense mechanism against competing fungi to protect and maintain ownership of its resources (Robinson, 2017). Zone lines can also form in response to changes of moisture content in wood and atmospheric conditions such as presence of increased CO_2 (Van Court and Robinson, 2019). Contrary to the wood degrading effects of bleaching, white-rot fungi create zone lines by plugging in the lumina of wood cells and secreting melanin with their hyphae (Tudor et al. 2014). Zone lines can offer additional structural support when melanin is secreted extracellularly. Melanin is a high molecular weight polymer so when it fills the pit membranes and lumina of adjoining wood cells, it helps form a complete and continuous, almost impenetrable, closely packed barrier against rival fungi (Morris, et al., 2021). In this way, zone lines can lead to a strengthening of wood.

Unlike the wood-decaying properties of white-rot basidiomycetes fungi, ascomycetes are soft-rot fungi that selectively metabolize cellulose and some lignin compounds from the secondary wall of plant cells, leaving the majority of structural components intact. To do so, hyphae tunnel inside lignified cell walls resulting in the formation of minute cavities (Gutierrez, 2016; Morris, 2021). Since ascomycetes fungi can't produce the enzymes to fully degrade cell walls, they must resort to secondary metabolites, such as pigments, for their continued survival. Some ascomycetes fungi can also produce zone lines that can be black or appear red, orange, green, or purple (Coates, 1984; Robinson, 2016).

Pigmentation, the last pattern type seen in spalted wood, results from the broad secretion of extracellular pigments by a small group of soft-rot ascomycetes fungi belonging to the Helotiales order (Gutierrez, 2016). Pigments are secreted as secondary metabolites produced when a supply of essential nutrients decreases or when the fungus needs additional protection from unfavorable environmental conditions (Pagano, et al., 2015). Pigments can also contribute to zone lines and one fungal species can produce a mixture of several different colored pigments (Pagano, et al. 2015). Pigments facilitate fungal survival by providing additional advantages such as light and ultraviolet resistance, bacterial and insect resistance, repelling competing rival fungal hyphae of another species, and prevention of desiccation in extreme environmental conditions (Giesbers, et al., 2019; Pagano, et al., 2015).

The pigments produced by fungi are biological pigments which are molecular compounds produced by living organisms. Color is owed to the presence of chromophores within the molecular structure of these compounds which selectively absorb and reflect wavelengths through their conjugated double bonds. The absorbed energy leads to electron excitation and changes in orbital occupancy, local bonding, and charge distribution. The non-absorbed light is

transmitted and/or reflected and registers as color to the eye (Gmoser, et al., 2017). The visible pigment compounds produced by fungi can be classified into four broad categories: carotenoids, melanins, polyketides, and azaphilones (Lin and Xu, 2023) (fig. 6).

Carotenoids produce hues of yellows, oranges, and reds. They are aliphatic polyene chain molecules. Carotenoids are powerful antioxidants and protect the fungi against harmful UV radiation and light photooxidation (Gmoser, et al., 2017). In collection materials, however, carotenoid-based pigments in feathers are known to be very light sensitive (Reidler, et al., 2014; Pearlstein, et al., 2015). Melanins produce hues of brown, black, grey, or dark green. These pigments are negatively charged hydrophobic aromatic heterocyclic organic compounds. Melanin pigments impart to the fungi antioxidant, antiviral, cytotoxic, thermo-regulatory, optical, electronic, and even radio/photoprotective properties (Meruvu, 2021).

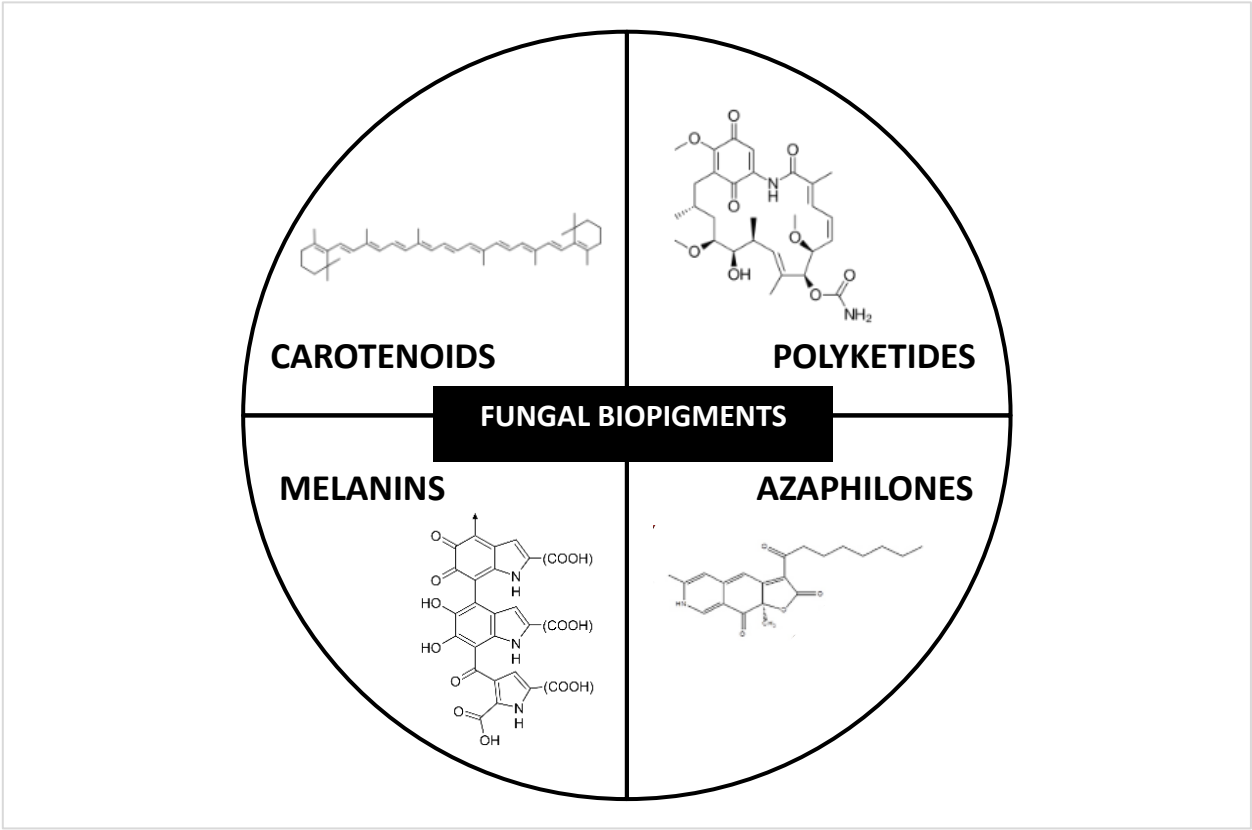


Figure 6: The four classes of biological pigments secreted by fungi and their molecular structures.

Polyketides are a large family of complex lipid molecules which react to form compounds of varying structures and biological abilities. Polyketide-based pigments can be a variety of shades depending on the structure of the resulting molecule which are subdivided into classes as anthraquinones, hydroxyanthraquinones, naphthalenes, naphthoquinone, and flavonoids to name a few (Caro, 2017). Azaphilone pigments are polyketide-derivative molecules that are structurally diverse and can range in shades of red, yellow, orange, green and brown (Caro, 2017). Polyketide pigments can promote fungal growth, provide resistance to environmental hazards such as desiccation, extreme temperatures, irradiations, and photo-oxidation, and enhance ecological interactions with other organisms (Meruvu, 2021).

2.2 Fungal Pigment Production and Biochemistry

Scientific research into spalting pigments was largely spearheaded in 2007 by Dr. Seri Robinson, a professor of wood anatomy and head of the Applied Mycology Lab within the Department of Wood Science and Engineering at Oregon State University. They are also a bio-artist working primarily with spalted wood. Robinson pioneered the study of fungal pigments by discovering a successful way to extract pigments from spalting fungi and to artificially induce spalting in the span of ten days, a process which previously took up to two years in the wild.

The process begins by growing varying species of spalting fungi on 2% malt extract agar (MEA) plates (fig. 7). Aggressive pigment production is induced by these researchers by incorporating finely chopped and sterilized, white-rotted maple wood, a key food source for spalting fungi, into the MEA plates (Giesbers, et al., 2019, 2021). The fungi are allowed to grow for 2-3 weeks before they are dehydrated in a fume hood for 24 hours. The dried fungi cultures

are then cut into pieces and placed into a vial of dichloromethane (DCM) which effectively extracts the pigments from the fungi. The solution is then filtered into glass vials to remove other solutes and contaminants to produce a solution of pure pigment suspended in solvent.

To date, the mycology lab successfully extracts and sells four pigments from three fungal species. These include *Chlorociboria aeruginosa* which produces a blue-green pigment *Scytalidium cuboideum* which produces a red pigment, and *Scytalidium ganodermophthorum* which produces both a purple and yellow pigment depending on the maturity of the fungi upon pigment extraction. The chromatic difference in pigment produced by these fungi can vary between plates, even within the same strain.

To ensure color consistency amongst the pigments sold by the mycology lab, the color of an extracted pigment solution is standardized to a lab-determined set of CIELab color measurements (table 1). As the purple pigment is a recently developed offshoot color from

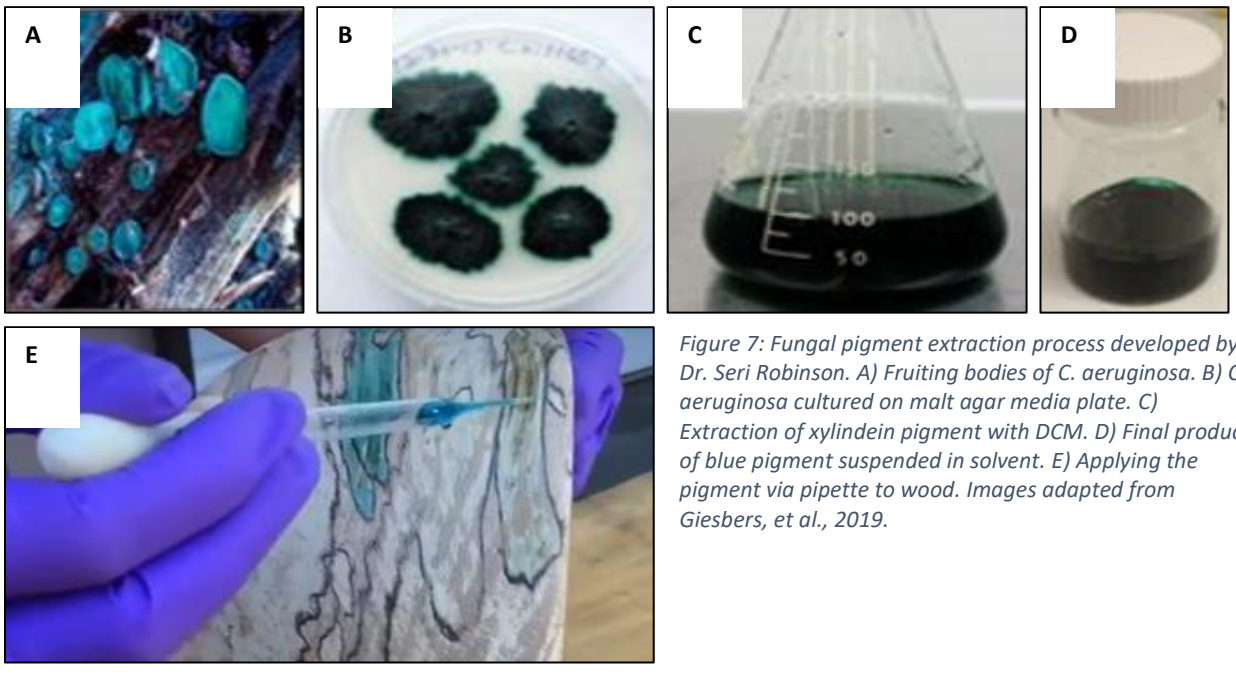


Figure 7: Fungal pigment extraction process developed by Dr. Seri Robinson. A) Fruiting bodies of C. aeruginosa. B) C. aeruginosa cultured on malt agar media plate. C) Extraction of xylindein pigment with DCM. D) Final product of blue pigment suspended in solvent. E) Applying the pigment via pipette to wood. Images adapted from Giesbers, et al., 2019.

S. ganodermophthorum, the lab is still in the process of establishing a standard for it. These values were met by concentrating or diluting extracts until the L* a* b* values were achieved (Robinson, et al., 2014b). Afterwards, the DCM is allowed to volatilize completely and since the pigments do not exist in powder form, they bind to the interior walls of the glass vials instead of forming collectable particulates. These colorants will need to be resolubilized by the consumer in order to use them as coloring compounds (Giesbers, et al., 2019, 2021). The extracted pigment can then be applied to wood via pipette to mimic the look of spalting. Alternatively, mature fungi samples from petri dishes can be used to inoculate wood and induce the natural spalting process.

It should be noted that as extracted pigments, these colorants are nonviable compounds and do not pose pathogenic or toxic risks, however, DCM is a problematic and unsafe solvent because it is known to be a potential carcinogen, mutagen, and a known greenhouse gas (Hinsch, 2018). Past studies have tried to find successful alternative solvents and pigment carriers such as oil, however, DCM has been demonstrated to be the most effective solvent by far for using extracted fungal pigments as a colorant carrier (Pittis, et al., 2018; Hinsch, 2018). A less hazardous carrier would be needed if these pigments were to be widely utilized in conservation.

Table 1: CIELab color values and swatches of fungal pigments.

	Chlorociboria aeruginosa	***Scytalidium cuboideum***	***Scytalidium ganodermophtherum***	
Pigment	Blue- Xylindein	Red- Draconian Red	Yellow- Unknown	Purple- Unknown
CIE L*a*b* color swatch				N/A
CIE L*a*b* Target values	L* 82.28, a* -11.06, b* -5.40	L* 82.32, a* 26.84, b* 13.19	L* 95.46, a* -3.00, b* -8.15	N/A

Extracted fungal pigments are known to adhere well to a variety of substrates (Gutierrez and Robinson, 2017). When viewed microscopically, the extracted pigments have a surface attachment to the wood, and they do not penetrate the wood cell wall as they would when secreted by fungi. In fact, although not confirmed, it is possible that fungal pigments adhere to wood similar to the way adhesives do; that is by hydrogen bonds and mechanical interactions (Gutierrez and Robinson, 2017). Studies have shown that, when applied as extracted pigments, the pigment tends to accumulate in the inter-vessel walls, pits, and helical thickenings of cells (fig. 8a-c). Pigment adherence on a substrate, rather than secreted impregnation of a substrate, might have implications for the lightfastness of the colorants. The different molecular structures and morphologies of each pigment may also play a role.

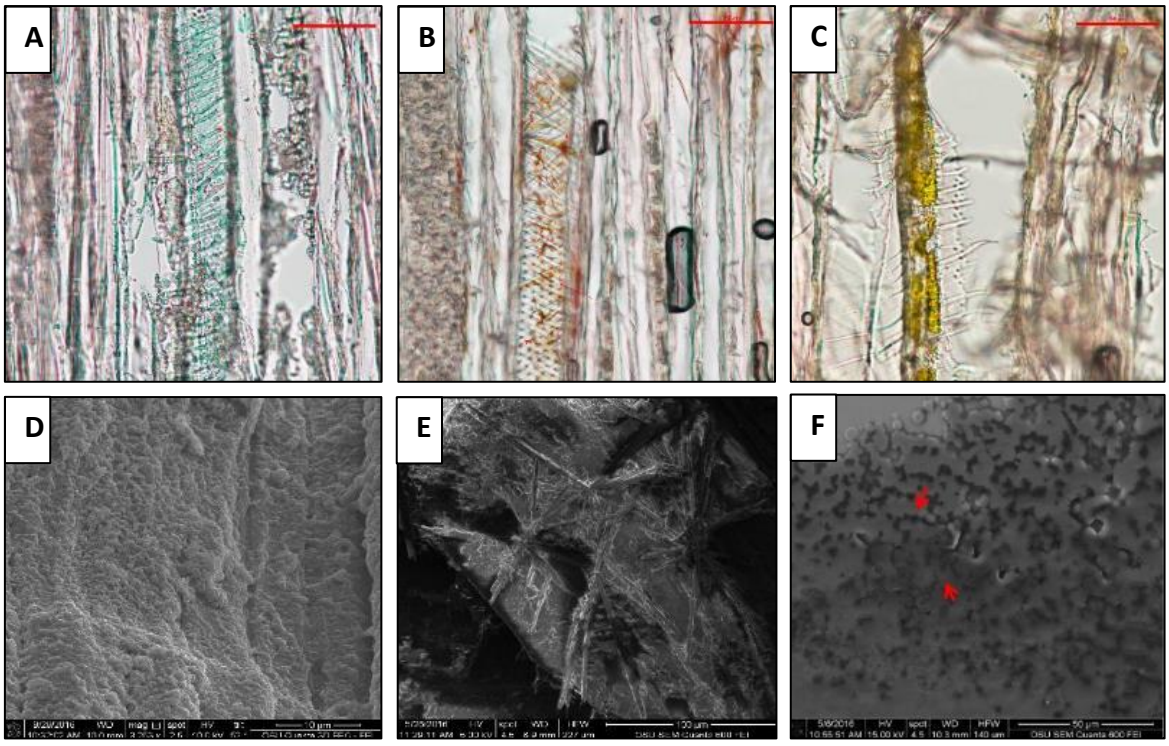


Figure 8: Morphology of three fungal pigments C. Aeruginosa (left), S. Cuboideum (middle), and S. Ganodermophthorum (right). A, B, and C show microscopic deposition of pigments in vessel cells of cottonwood at 20X magnification. Bottom are SEM images showing pigment morphology on wood. D) C. Aeruginosa forms an uneven amorphous film. E) S. Cuboideum forms flower-like crystal structures on wood. F) S. Ganodermophthorum has a spongy amorphous texture on wood. Images adapted from Gutierrez and Robinson 2017.

Chlorociboria aeruginosa

Chlorociboria aeruginosa produces a blue-green pigment identified as xylindein which falls under the polyketide umbrella of fungal pigments (fig. 9). This pigment has a unique nonpolar perixanthenoxanthene (PXX) core structure which is responsible for its distinctive morphology, bonding capabilities, and light stability in two key ways: Firstly, the structure allows for high levels of intermolecular and intramolecular hydrogen bonding. Secondly, the adjacent aromatic rings allow for π-stacking (Harrison, et al., 2017). These structural features enable the molecule to delocalize electron charge and dissipate energy upon photoexcitation, qualities which contribute to the photostability of these pigments (Giesbers, et al., 2019). When not suspended in solvent, xylindein has an amorphous structure and deposits on surfaces in smooth but porous and inhomogeneous films (Gutierrez and Robinson, 2017) (fig. 8d).

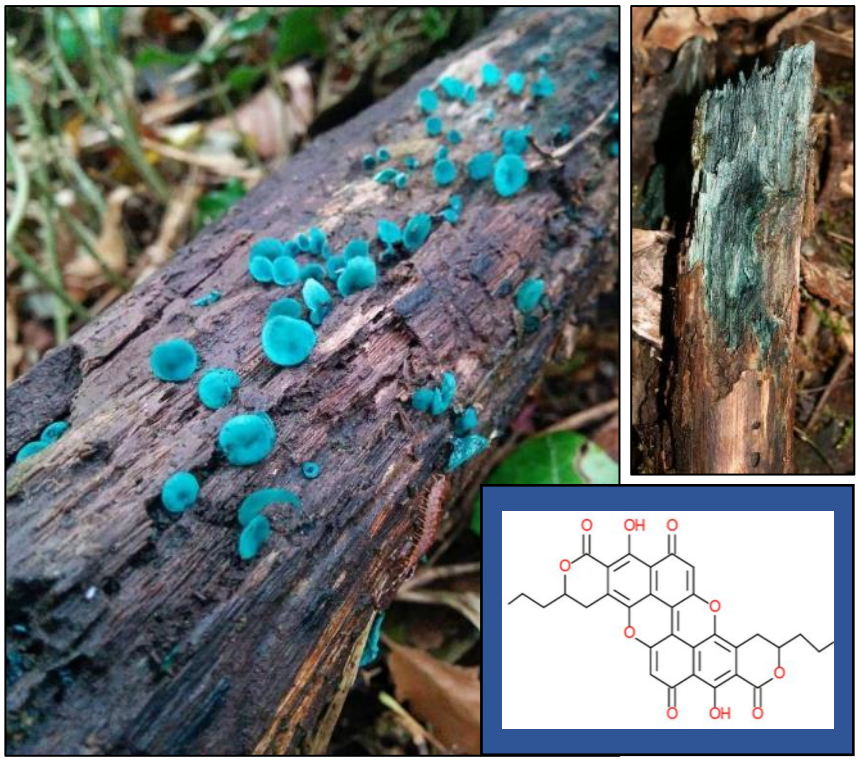


Figure 9: C. aeruginosa fruiting bodies (left), interior or spalted wood exhibiting blue pigmentation (top right), and the xylindein perixanthenoxanthene structure (PXX) (bottom right). Images adapted from Robinson, et al., 2012.

Scytalidium cuboideum

The pigment produced by *Scytalidium cuboideum* is known as "Draconian Red" which can range in shades of reds, pinks, and oranges depending on concentration (fig. 10a, b). This pigment is unique amongst the spalting fungi because it is the only one thus far identified to have a defined crystalline morphology. The pigment has a polyketide-derived naphthoquinone composition similar to quinone dyes like madder (Dulo, et al., 2021) (fig. 10d). Draconian Red was characterized as a novel organic crystal which forms needle-like longitudinal compounds that crystallize into flower-like structures (fig. 8e, 10c). These crystals are highly stable due to their strong intermolecular bonding (Gutierrez and Robinson, 2017).

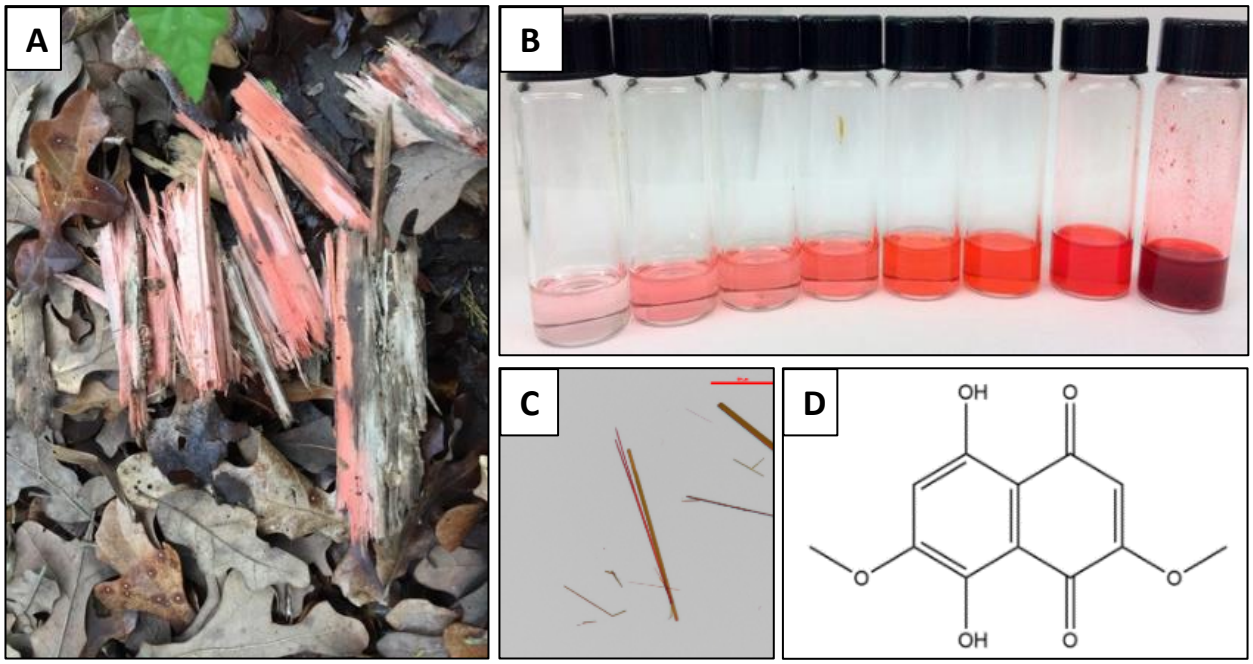


Figure 10: A) Wood spalted with S. cuboideum. B) Range of colors of pigments from S. cuboideum suspended in solvent. C) Needle-like crystals of the pigment in two colors, red and orange. D) Naphthoquinone PXX molecular structure of pigment. Images adapted from Gutierrez and Robinson 2017.

Scytalidium ganodermophthorum

Scytalidium ganodermophthorum is responsible for both the yellow and the purple colors studied in this research (fig. 11). The difference in color is dependent on the maturity of the fungi upon extraction. *S. ganodermophthorum* produces a yellow pigment by week seven, green by week 13, and finally purple by week 30. During these transitions, the color can appear brown or gray. This might be due to the potential ability of *S. ganodermophthorum* to produce multiple pigments that mix in solution although the ability to produce multiple pigments has thus far not been recorded in other spalting fungi. The colors produced might also be affected by pH, polarity, and by pigment concentration (Gutierrez, et al., 2021). The structure of the pigment from *S. ganodermophthorum* has not been characterized, however, studies have shown that when deposited as an extracted pigment onto wood, it forms a thick and bumpy sponge-like texture with no characteristic shapes (Gutierrez and Robinson, 2017; Van Court and Robinson, 2019).

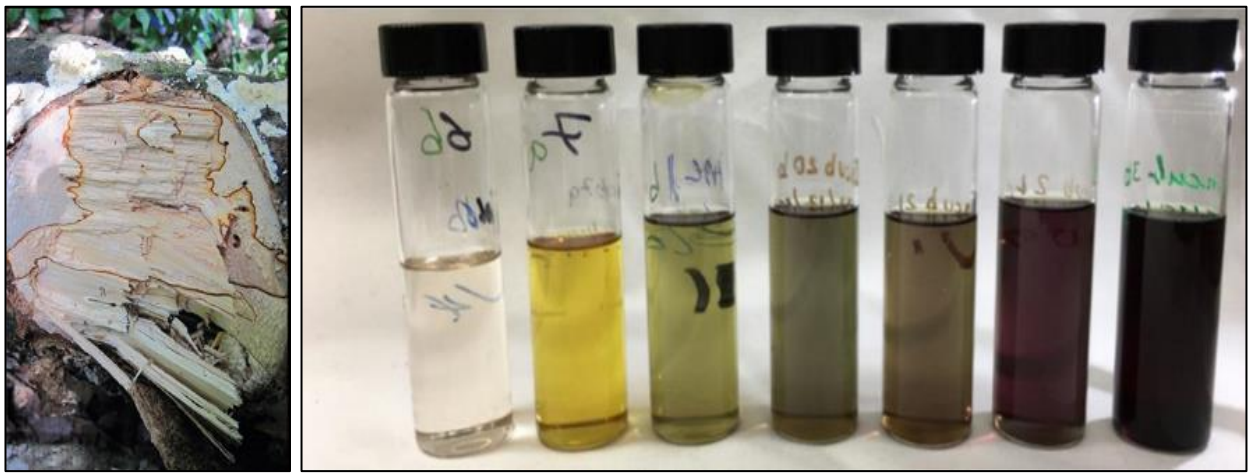


Figure 11: Wood spalted with S. ganodermophthorum (left). Extracted pigments from S. ganodermophthorum suspended in solvent (right-Image from Robinson 2017).

3. Experimental Design: Accelerated Light Aging and Color Measurement

3.1. Materials and Sample Preparation

Four fungal pigments extracted from *Chlorociboria aeruginosa, Scytalidium cuboideum,* and *Scytalidium ganodermophthorum* were sourced from the Applied Mycology Lab at Oregon State University and delivered by mail to the UCLA/Getty conservation research lab at the Getty Villa Museum in Los Angeles California where the experiment took place. A total of 32 fungal pigment coupons, divided into four sets, were made for the accelerated aging experiment. One set of 8 coupons includes each of the four pigments on paper and each of the four pigments on wood. One set served as a control while the other three sets underwent accelerated aging. To prepare the wooden coupons, a sheet of 0.35-inch-thick maple wood veneer was cut into ¼ inch by 2.0-inch rectangles. All surfaces were lightly sanded and dusted prior to being pigmented. The paper coupons were prepared by cutting Grade 1 circular 100% cellulose Cytiva 1001125 Whatman™ Qualitative Filter Paper into 2.0 by 2.0-inch squares.

The fungal pigments were prepared by resolubilizing them in their original glass vials with 20 ml of DCM. The pigments were not standardized to OSU's CIELab values because a deeper saturation level was desired. Since the experiment seeks to measure the amount of color change after light aging, starting with the highest saturation level possible would increase the measurable amount of color change. Fungal pigments were applied to all coupons via pipette. This was achieved by placing all coupons atop silicone release mylar and applying 5 drops of pigment to completely cover each surface. To get a deeper saturation, each coupon received three total coats of pigment and was allowed to hang dry for 30 minutes between coats.

After the coupons were prepared, they were set into Q-Lab specimen mounts according to color (fig. 12). Each mount has two 43 x 96 mm (1.7 x 3.8 in) exposure windows which accommodated three coupons per window. The control set was grouped together and placed into a separate mount. All coupons were photographed to visually document the color before and after accelerated light aging with a Nikon D90 DSLR camera. All photos were white balanced, and exposure corrected until RGB values reached 200. The control set was placed into a dark sealed archival blue board paper box to ensure they would receive zero light exposure during the duration of the experiment. When not undergoing accelerated aging or color measurements, all other coupons were also kept in a dark sealed archival box to prevent natural light exposure.

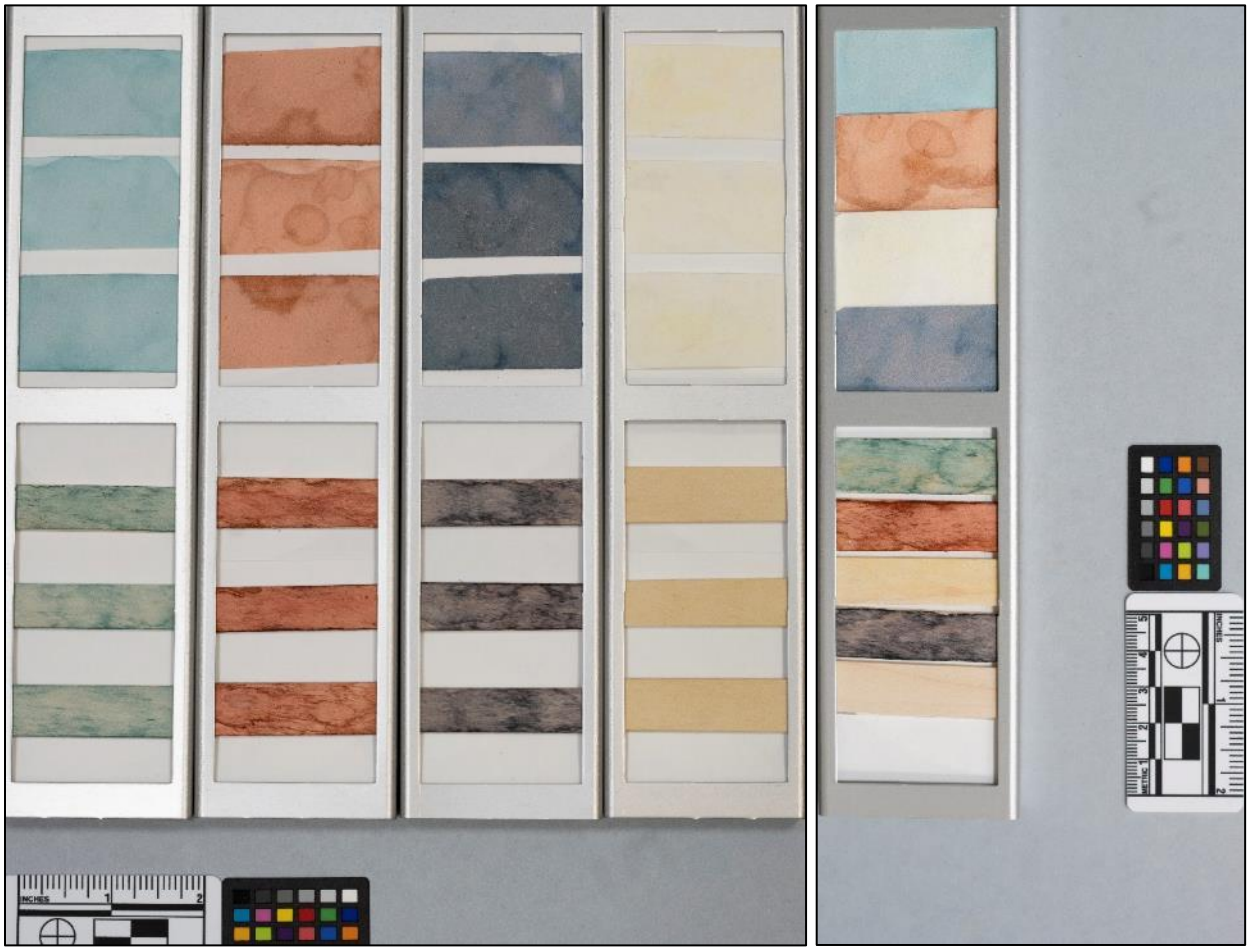


Figure 12: Prepared fungal pigment paper and wood coupons mounted by color. Three sets underwent accelerated aging (left), and one set was the control (right).

3.1.1. Visual Observations during Sample Preparation

To achieve an even distribution of color, the coupons were initially going to be painted by dipping them into the vials of fungal pigment. This application method, however, was ineffective because the high dissolution strength of DCM would strip the pigment from the substrate every time the coupons were lifted from the vial. For this reason, fungal pigments were instead applied to coupons with a pipette. Although care was taken to achieve an even distribution of pigment, pipette application did result in the formation of tidelines.

During pigment application, it was interesting to observe that the pigments had slightly different behavioral properties (fig. 13). The red pigment for example, had a drastic color change upon drying. When first applied, the pigment is a bright and vivid neon red but dulls down in seconds to a paler pinkish red. This pigment also spread across the surface of the substrates more quickly and easily than the other pigments. The red had the highest saturation ability as compared to the other pigments as the color would become deeper with each additional coat. There was not as much of a visual change in color saturation with additional coats of the other three pigments.

The blue pigment also dried to a softer color but did not start out as vivid as the red. The blue also did not spread as quickly and easily across substrate surfaces. The yellow pigment had the least coloring power and achieving a deeper saturation of color proved to be very difficult. It was found that blue and yellow pigments could be combined to produce green. The purple pigment was the most unique in behavioral properties. First, the pigment did not spread as quickly across the surface of a substrate but would rather penetrate deeper. This resulted in coupons with more tidelines due to the circular deposits of color. The purple pigment seemed to

be the most sensitive to surface variations as it would quickly gather at edges, creases, or crevices in the substrate. Most interestingly, the purple pigment had a distinct quality where it would slightly separate once deposited onto a paper substrate. The brighter purple components of the pigment would seemingly cluster at the edges of tidelines leaving much of the substrate a duller gray color. This behavior is also observed at the reverse of the paper coupons; the purple seeped through the depth of the paper and collected at the back which made the coupons appear more purple on the reverse than the obverse surfaces.

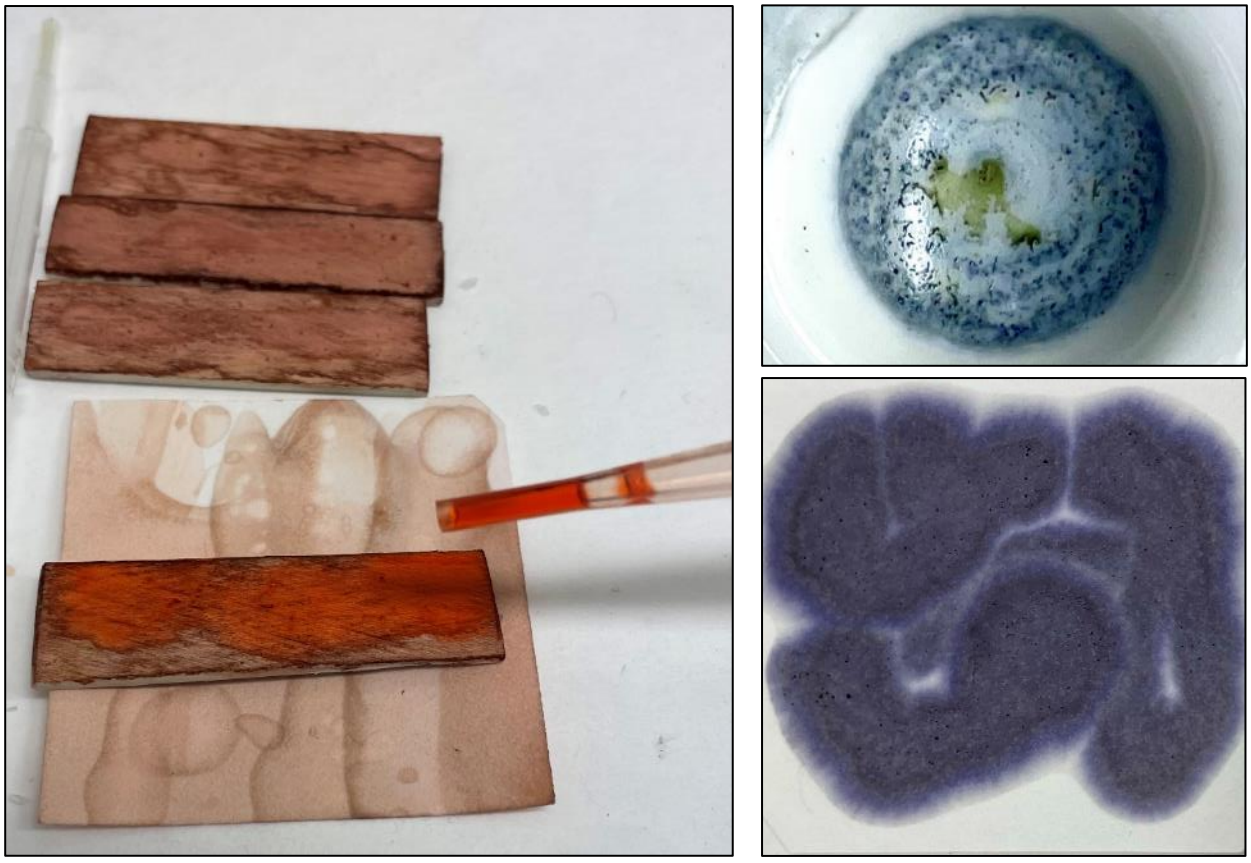


Figure 13: The red pigment appeared much more vivid when first applied but dulled down within a few seconds after the solvent evaporated (left). The yellow and blue pigments could be combined to produce green (top right) as seen here where both pigments were combined in a well in a ceramic paint palette. The purple pigment becomes the most concentrated at the edge of tidemarks where it appears as a truer purple (bottom right).

3.2. Methodology: Blue Wool Standards (ISO BWS)

ISO BWS cards, sourced from Talas, were half covered with aluminum foil. One card was placed inside the accelerated aging chamber alongside the fungal pigment coupons during the experiment. Another card was placed in the dark sealed archival box housing alongside the control set of fungal pigment coupons. All fungal coupons also had half their surfaces covered with aluminum foil to assess whether the slight increase in temperature maintained within the accelerated aging chamber would contribute to fading. At the end of the experiment, the foil was removed from both coupons and ISO BWS cards to assess the degree to which fading occurred.

3.3. Methodology: Reflectance Spectrophotometry

Reflectance spectrophotometry was used to quantitatively measure the color of each coupon. The measurements taken before and after accelerated aging were compared to determine the total color change incurred from the experimental conditions. Since the pipette application of fungal pigments created color variability and tidelines among the coupons, multiple measurements were taken along the surface of every coupon which were later averaged. Limited by the spectrophotometer's 4 mm aperture size, the paper coupons allowed for six measurements to be taken while the wood coupons allowed for four measurements. Stencils were created to ensure that locations of color measurements taken before aging matched exactly with those taken after aging. Stencils were made from filter paper with 4 mm holes cut out for the exposure areas.

Color readings of each coupon were spectrophotometrically measured with an X-Rite Ci64 Handheld Spectrophotometer manufactured by Pantone. The instrument utilizes a gas-filled tungsten lamp + UV LEDs and has a measurement cycle time of 2 seconds. The instrument

measures between 400 and 700 nanometers and was set to capture in reflectance mode. Measurements were taken using the 4mm aperture. The instrument was calibrated before each measurement campaign with X-Rite's Calibration Reflection Standard which utilizes a ceramic plaque for white measurements and a trap opening for black measurements. To take a measurement, the coupons were positioned flat on top of a sheet of filter paper. The filter paper stencil was slid in between the instrument and the coupons to ensure the correct placement of the aperture window but was removed to take a measurement. The data pulled from each measurement is automatically saved and uploaded into Pantone's Color iQC software, a proprietary color data management software also manufactured by Pantone.

Color measurements were captured as, and differences calculated from, CIE Lab values to detect total change in luminosity (ΔL*), total changes in shifts from red to green (Δa*) and total changes in shifts from yellow to blue (Δb*). The CIEDE2000 method was used to calculate the delta in color change, ΔE*. Updated and published in 2001, CIEDE2000 calculations improve upon those of 1976, which is simply the distance between the two colors on the chromaticity sphere, calculated using Euclidian geometry. CIEDE2000, the most complex algorithm thus far, solves incongruities in saturation and lightness, bringing the resulting ΔE* values closer to changes perceivable by the human eye (Sharma, G. 2004).

Six measurements were taken on each paper coupon and four were taken on each wood coupon. The before-treatment (BT) CIELab values were the calculated averages of all measurements per coupon (6 per paper coupon and 4 per wood coupons). Since half of the coupons were half-covered in foil and unexposed to light, only half of the measurements yielded significant color change. For this reason, the pigments were ascribed two ΔE* values: one for the light-exposed halves of coupons and one for the unexposed halves (3 per paper coupons and 2

per wood coupon). This was done because the inclusion of L*a*b* values from the unexposed sides would skew the data, resulting in an averaged value that didn't accurately represent the color change of the exposed halves.

3.4. Methodology: Accelerated Light Aging

As this study seeks to compare the light stability of fungal pigments relative to museum standards, the parameters for the accelerated aging experiment were set to mimic broad spectrum lighting conditions that could be found in a small museum or historic home without UV filtration. Another cycle of accelerated aging on new fungal coupons with UV filtration was intended to assess UV-specific changes to color, however, time and resources prevented this. Accelerated aging was conducted using a Q-Sun Xenon Test Chamber, model Xe-1-B, at the UCLA/Getty conservation research lab (fig. 14). The machine uses a xenon arc lamp to simulate full spectrum daylight ranging from ultraviolet through infrared, with emissions in the ultraviolet at around 365 nm and strong peaks around 400-500 nm and 600-700 nm. The light was controlled to a specific irradiance of 0.26 W/m^2 at 420 nm. Incident illuminance without a UV filter for this chamber is 20,600 lux with emissions in the ultraviolet at around 365 nm and strong peaks around 400-500 nm and 600-700 nm into the infrared. The temperature, recorded by the machine, was kept around 40° C. The chamber replicates the light conditions that an object would receive if placed near a window without UV filtration. The Q-lab specimen mounts, housing the three sets of both paper and wood fungal pigment coupons, were placed vertically within the accelerated aging chamber along with an ISO BWS. An unpigmented paper and

unpigmented wood coupon were also aged to assess color shifts of the substrates. The coupons were aged for a total of 24 hours.

Figure 14: Fungal coupon pigments and ISO BWS, all half covered with aluminum foil, in the Q-Sun Xenon Test accelerating aging chamber.

4. Results

The results of this study are extrapolated from the average color change (ΔE*) of fungal pigment coupons after accelerated aging. The data is summarized into a series of charts that graph the CIE L* a* and b* values of paper and wood coupons taken before and after the aging experiment. Trends in increasing or decreasing luminance, shifts to green or red, and shifts to yellow or blue are described in section 4.2 and associated graphs. Values for the control set of

coupons are graphed alongside those that underwent accelerated aging so that comparisons could be made. For ease in discussion, coupons pigmented with *C. aeruginosa* will be referred to as blue coupons, coupons pigmented with *S. cuboideum* will be referred to as red coupons, and coupons pigmented with *S. ganodermophthorum* will be referred to as purple or yellow coupons, correspondingly.

4.1 Accelerated Light Aging: Blue Wool Standards

Museums generally strive to limit light exposure of materials with medium sensitivity to 150 lux. Assuming that a museum is lit 8 hours a day, 6 days a week, 52 weeks a year, we calculated an exposure of about 436,800 total lux hours over the course of a year. Using the reciprocity principle discussed earlier, a full year of lux exposure can be simulated in the accelerated aging chamber in 19 hours (436,800/22,600). Cumulatively, based on the lux measured in the chamber and the number of hours for which the samples aged (22,600 lux for 24 hours), the samples were exposed to a total of 542,400 lux of full spectrum light without UV filtration. This simulates 16 months of light aging in real-time if we extrapolate based on parameters referenced above.

After 24 hours in the accelerated aging chamber, the ISO BWS #5 showed signs of just-noticeable fading (fig. 15). Since the targets on an ISO BWS card fade at known ranges within estimated rates, the degree of fading observed can be roughly quantitated to the total light dose which they have received. With UV exposure, the dose to initiate just-noticeable fading in ISO BWS #5 is 8 Mlx h (Michalski, 2017). This translates to an exposure of 333,333 lux (8 Mlx h/ 24 hours) at minimum for fading to begin. The dose for the next ISO BWS #6 is 20 Mlx h which

translates to 833,333 lux. As the accelerated aging chamber put out an estimated total of 542,400 lux of full spectrum light, the level and degree of fading observed on the ISO BWS accurately correlates.

ISO BWS #4-6 are in the category of medium sensitivity and if exposed to 150 lux without UV filtration, colors in this category would almost completely fade in 200-7,000 years (Michalski, 2017). Aniline synthetic dyes, historic dyes and most colorants from plant extracts and insects (lac, cochineal) fall in the category of high sensitivity, ISO BWS #1-3 (CIEE, 2004). If exposed to the same illuminance of 150 lux without UV filtration, colors in this category would almost completely fade in 6 months to 7 years (Michalski, 2017).

Since all the fungal pigment coupons tested showed high signs of fading, they cannot be described as medium sensitive materials. The red pigment on paper did retain considerably more color than the other pigments, however, fading was still observed. This study established that the fungal pigments tested undergo considerable light and UV-induced color change equivalent to 16 months at 150 lux plus UV. Since the coupons weren't removed from the chamber earlier than 24 hours, the initial onset of fading was unfortunately not detected. Future accelerated aging experiments should prioritize assessing fading at regular short intervals to define the lightfastness of fungal pigments relative to ISO BWS more precisely. Future aging experiments should also incorporate UV filtration to remove the variable of UV-induced degradation from the assessment of lightfastness. The results of this experiment, however, can confirm that, by museum standards, extracted spalting fungal pigments are generally of high sensitivity and not more lightfast to other comparable biological colorants. No visible fading was noted in the ISO BWS card that was kept alongside the control set of coupons in the lidded box. The control set of fungal pigment coupons also did not exhibit perceivable fading.

4.2 Accelerated Light Aging: Color Measurements

There averaged BT and PT values were used to calculate the delta in color change (ΔE*) per pigment (graph 1). The ΔE* was also calculated for the control set of coupons as well as an unpigmented paper and unpigmented wood coupon (graph 2). The calculated averages were also used to calculate shifts in lightness, red/green, and blue/yellow (table 2).

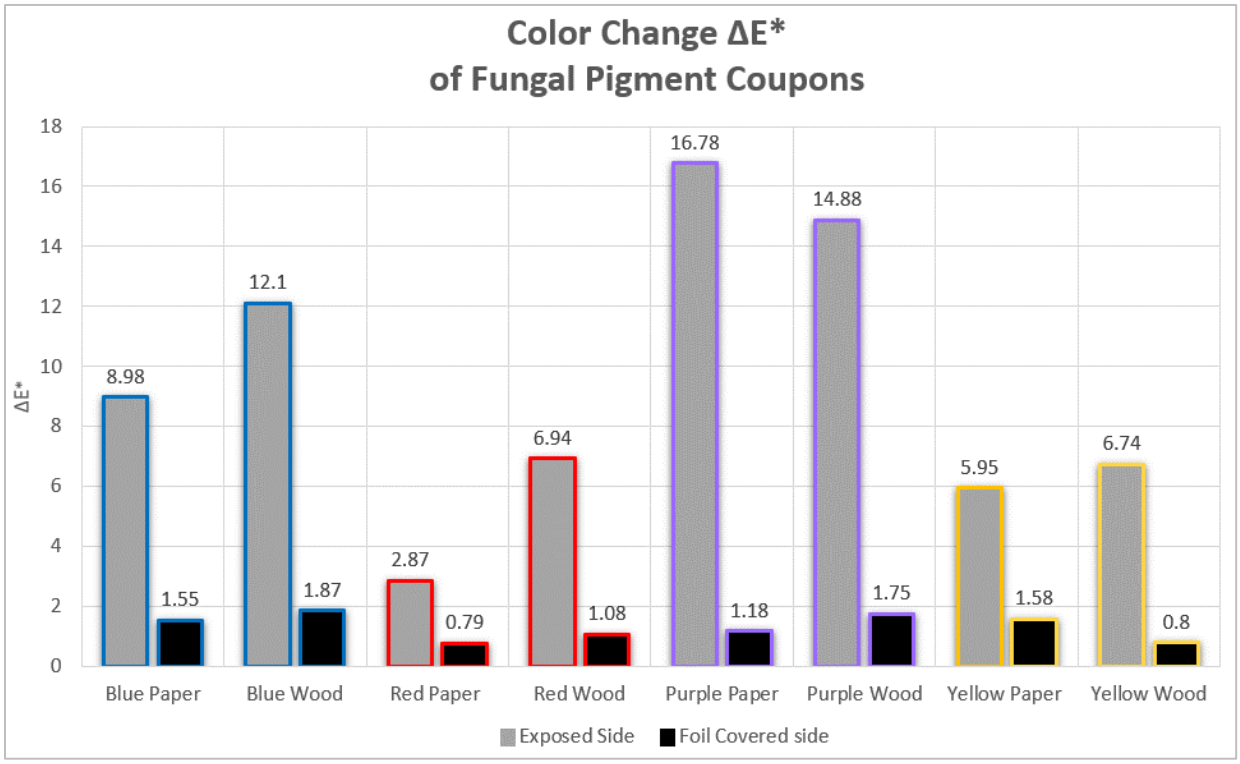
The ΔE* for blue paper and wood coupons was 8.98 and 12.1, respectively. CIELab values indicated that the paper coupons became overall lighter and shifted towards red and yellow. Wood coupons became darker but also shifted towards red and yellow (graph 3, fig. 16). The ΔE* for red paper and wood coupons was 2.87 and 6.94, respectively. All coupons became lighter and shifted towards green, however the paper coupons shifted towards blue whereas the wood coupons shifted towards yellow (graph 4, fig. 17). ΔE* for purple paper and wood coupons was 16.78 and 14.88, respectively. All coupons became lighter and shifted towards red and yellow (graph 5, fig. 18). Yellow paper and wood coupons had a ΔE* 5.95 and 6.74, respectively. All coupons became lighter and shifted towards red, however, the paper coupons shifted towards blue whereas the wood coupons shifted towards yellow (graph 6, fig. 19).

Table 2: Assessment of light and color shifts calculated from averaged post-aging CIELab values.

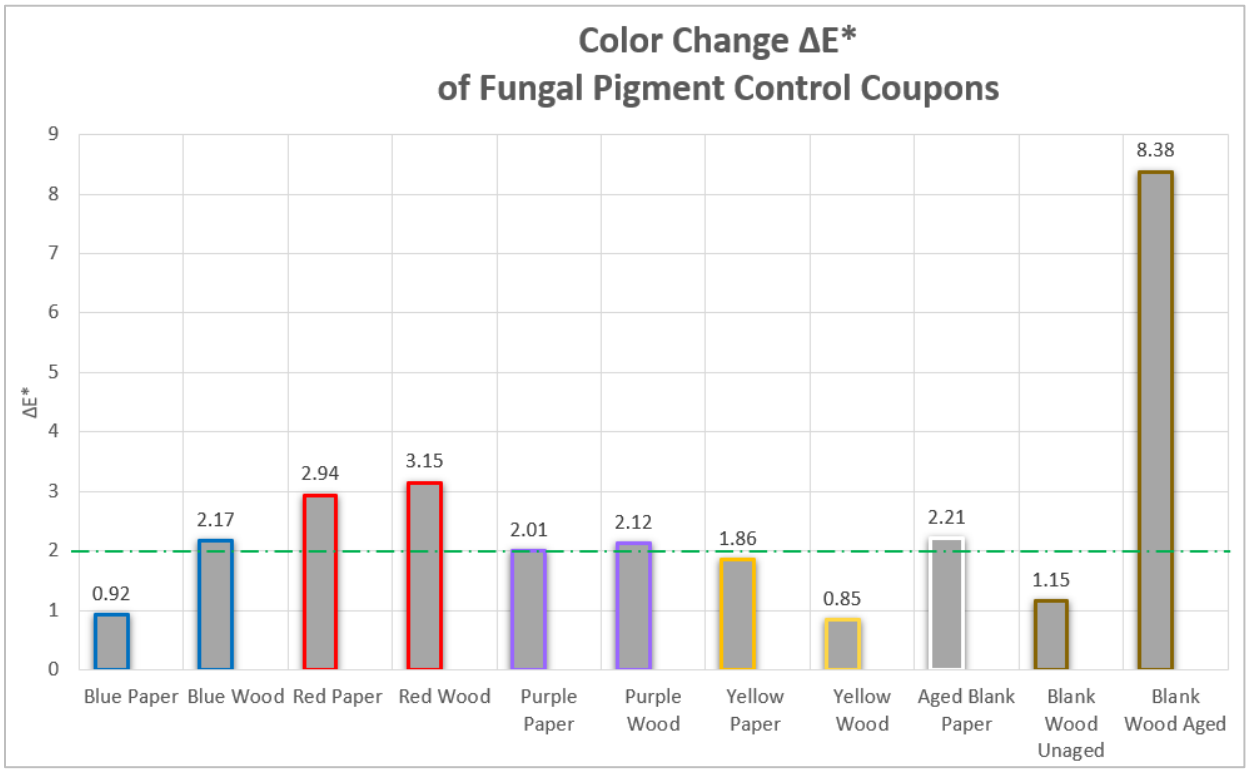
	***C. aeruginosa* -Blue-**		***S. cuboideum* -Red-**		***S. g.* -Purple-**		***S. g.* -Yellow-**	
	Paper	Wood	Paper	Wood	Paper	Wood	Paper	Wood
ML*	+7.06	-2.90	+2.36	+1.04	+17.08	+10.85	+2.81	-5.97
	Lighter	Darker	Lighter	Lighter	Lighter	Lighter	Lighter	Darker
Ma*	+7.51	+7.95	-2.36	-5.88	+6.16	+4.70	+2.21	+5.44
	Redder	Redder	Greener	Greener	Redder	Redder	Redder	Redder
Mb*	+2.72	+10.48	-1.65	+6.04	+1.66	+13.68	-6.93	+4.18
	Yellower	Yellower	Bluer	Yellower	Yellower	Yellower	Bluer	Yellower

Figure 15: Fungal pigment coupons, post aging. Foil has been removed from the left half of all surfaces as well as from the right side of the ISO BWS card, the latter which exhibits just noticeable fading at target #5. Below the ISO BWS card are a blank paper coupon and a blank unpigmented wood coupon that were also aged to assess color shifts of unpigmented substrates. Severe darkening and yellowing of the blank wood are clearly evident on the right, light-exposed, sides..

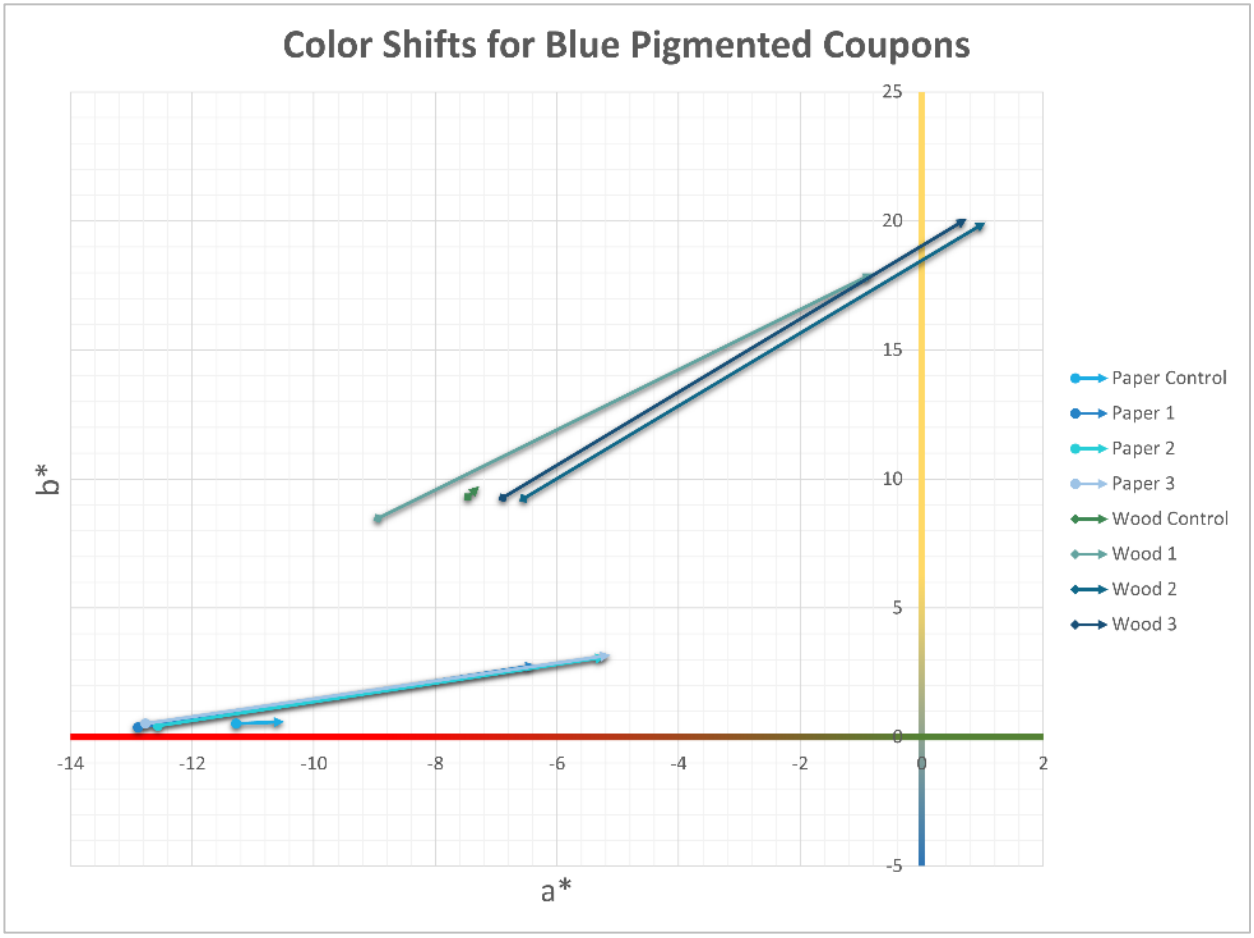
Graph 1:



Graph 2:



Graph 3:



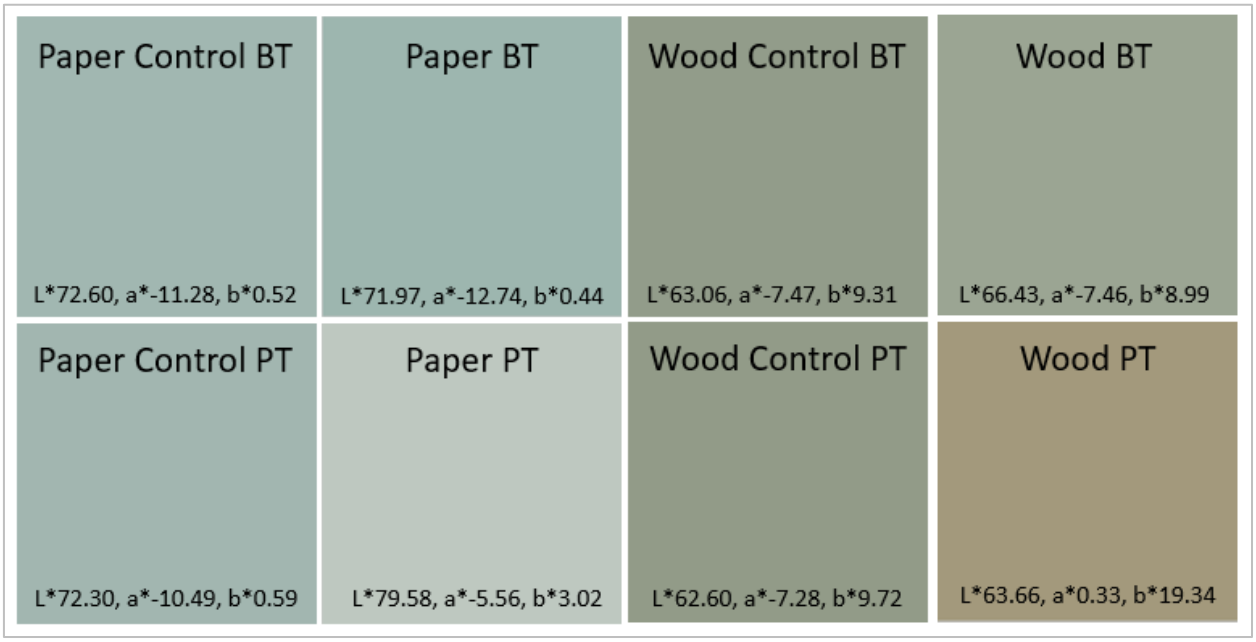


Figure 16: CIELab color swatches from averaged L a* and b* values of fungal pigment coupons pigmented with C. aeruginosa.*

Graph 4:

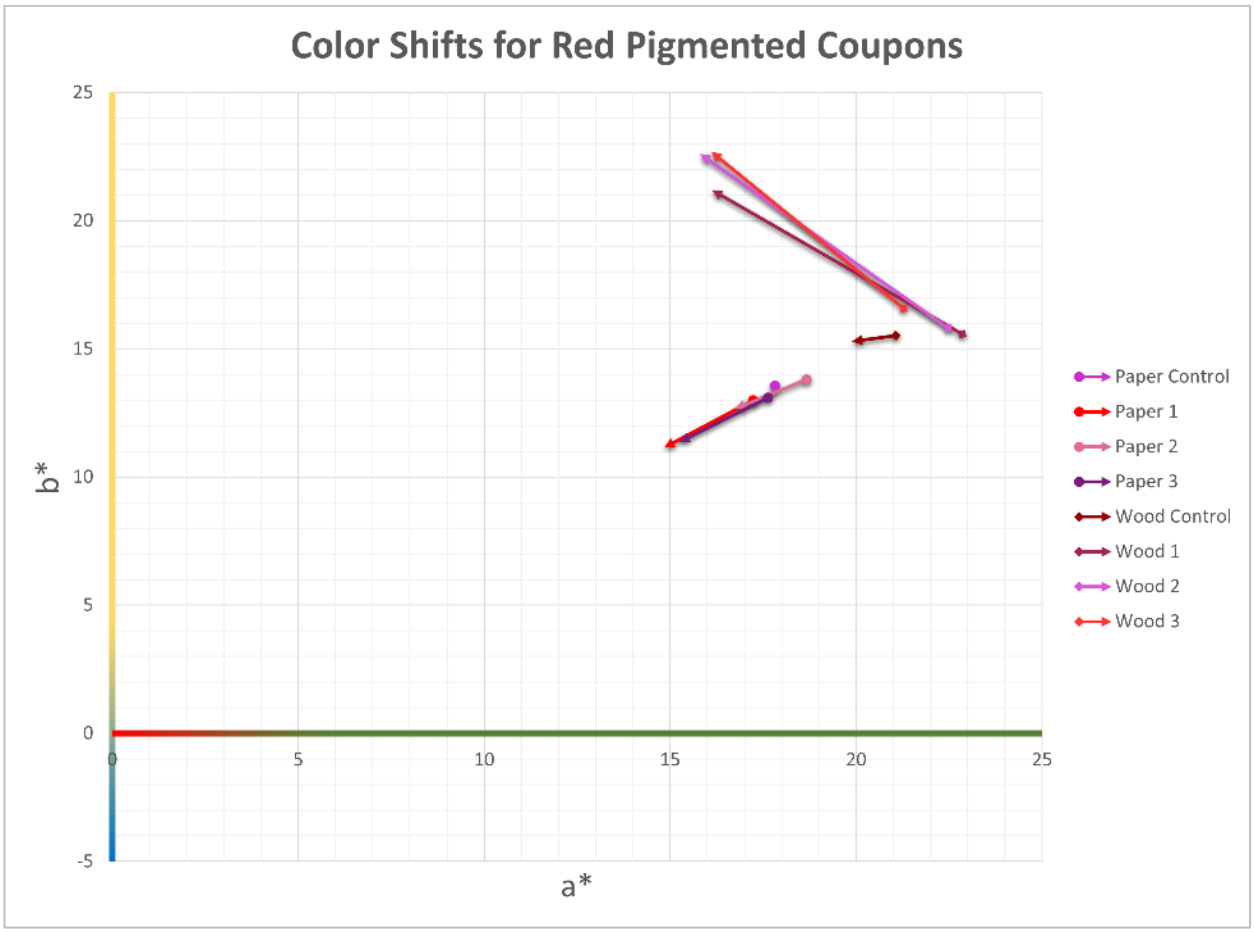
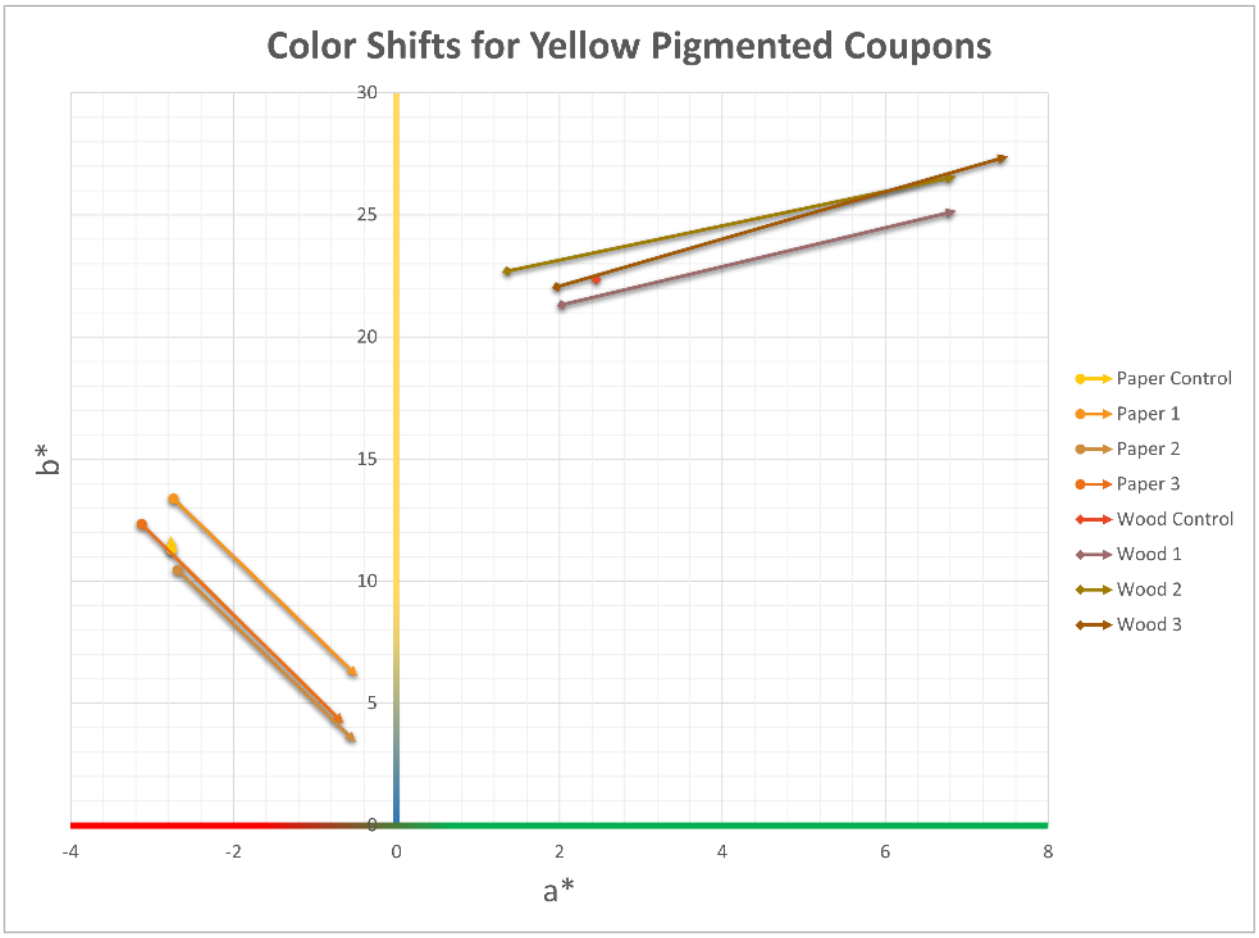


Figure 17: CIELab color swatches from averaged L a* and b* values of fungal pigment coupons pigmented with S. cuboideum.*

Graph 5:

Figure 18: CIELab color swatches from averaged L a* and b* values of fungal pigment coupons pigmented with S. G.*

Graph 6:



Paper Control BT L*86.73, a*-2.76, b*11.28	Paper BT L*86.17, a*-2.85, b*12.05	Wood Control BT L*75.08, a*2.45, b*22.35	Wood BT L*75.64, a*1.79, b*22.02
Paper Control PT L*85.41, a*-2.77, b*11.85	Paper PT L*88.99, a*-0.54, b*4.56	Wood Control PT L*74.79, a*2.45, b*22.15	Wood PT L*70.18, a*7.08, b*26.38

Figure 19: CIELab color swatches from averaged L a* and b* values of fungal pigment coupons pigmented with S. G.*

5. Interpretation and Discussion

The human eye can only detect a ΔE* of 2.0 or higher; anything smaller than 2 is visually insignificant to human perception (Hinsch, 2015). The control set of coupons that had a ΔE* of 2.0 or lower included blue on paper, purple on paper, yellow on both paper and wood, and blank unaged wood. The rest had ΔE* of around 3 or below. Although minimal, these values do illustrate that even in completely dark conditions, the pigments did have statistically measurable, yet visually insignificant value shifts. These were not induced by light but perhaps to slightly elevated temperatures within the chamber produced by the high light levels.

Overall, the pigments on wood yielded higher ΔE* values than on paper except for purple where colorants on paper surpassed wood by only 1.9. These results suggest that fungal pigments are more lightfast on paper than they are on wood. This was surprising considering that wood is the natural substrate for these pigments in the wild, and pigmented wood in intarsia has retained color for hundreds of years. Extracted pigments, however, are known to deposit differently on wood when applied by hand as opposed to when secreted by fungi. In a study that looked at the microscopic deposition of these pigments on wood applied via pipette, the main distribution of extracted pigments was found in the vessels (Gutierrez, 2017). This contrasts with naturally secreted pigments which concentrate in wood ray cells where simple sugars are located (Tudor, et al., 2013). In fact, this study showed that the extracted pigments have a surface attachment to the wood and don't penetrate the cell wall (Gutierrez, 2017). It may be likely that the surface deposition of secreted pigments on wood limits their physical bonding within internal wood structures, as would occur during fungal secretion.

Even more, the properties of the solvent carrier, DCM, could also play a contributing role to the surface deposition of pigments. To start, DCM has a boiling point of 39.5° C and has a very high evaporation rate that limits the penetration capacity of the pigments to deposit in deeper internal structures (Pittus, et al., 2018). As the paper coupon substrate is much thinner and more porous than the wood coupon substrate, it is possible that the fungal pigments suspended in DCM were able to penetrate the paper more efficiently than they did on wood. This deeper and more thorough penetration of the pigments into the paper might increase the bond strength that accounts for the higher degree of lightfastness observed. DCM moves through material primarily through diffusion rather than capillary action due to its high chemical potential as a solvent with extremely high vapor pressure, low boiling point, major concentration gradient, and polar aprotic nature. This means that diffusion of DCM through the wood will bring the pigmented solvent in direct contact with components of the cell wall such as lignin, hemicellulose, and other wood extractives which can hinder rate of diffusion (Robinson, et al., 2014a).

Thermodynamic equilibrium between these cell components and the pigmented solvent can also affect the penetration of pigment deposition and the resulting coloring capacity. As an organic solvent-solute solution, the pigmented DCM is not in a fixed state but is rather a constantly forming and disassociating mix of complexes that struggle to maintain thermal equilibrium conditions. This is disrupted when new solutes are introduced, as would potentially be the case when DCM is in contact with the wood cell components. The extractives, lignin, and hemicellulose profile in wood can affect both the number of solutes and the solute balance in the pigmented DCM, potentially rejecting the dye compounds to the wood matrix in favor of more thermodynamically favorable solutes (Robinson, et al., 2014a). These additional wood components also introduce molecular structures with varying polarities and affinities. As the

paper substrates utilized for the coupons in this experiment are composed of 100% cellulose, they are purer than wood in terms of chemical structure and polarity of the matrix. This might contribute to the superior penetration of pigmented DCM and subsequent colorfastness observed in paper coupons over wood coupons.

Alternatively, the extracted pigments themselves may preferentially bind to hemicellulose or lignin, and only moderately to cellulose due to the lesser availability for molecular bonding and interaction sites (Hinsch, et al., 2015). Although the paper coupons are statistically more colorfast according to their overall lower ΔE* values, the wood coupons appear to statistically retain higher saturation values for a* and b*. Although fading is generally associated with the visual lightening of a color, this doesn't solely correlate to the increase of the L* value. In the CIELab color space, 'lightness' is quantified as a percentage from absolute darkness (black) to maximum lightness (diffuse white). The a* and b* values, on the other hand, are direct measurements for saturation. In the sphere, the centermost point is gray and correlates to the least amount of saturation. The more removed from the center, the higher the saturation values. These higher saturation values, however, might be attributed to the color of the substrate itself. Unlike the white color of the substrate of the paper coupons, the wood coupons are already inherently yellow colored. This color might be contributing to the increased saturation values in wood coupons. The yellow substrate color of the wood can also influence the perceived color of the pigment on top. For example, the blue pigment on wood appears much greener than when applied on white paper. This could be attributed to the optical mixing of yellow wood and blue overlying pigment.

Also important to consider is the aging properties of wood vs paper and how that can contribute to perceived color and lightfastness of fungal pigment coupons. As opposed to

becoming lighter, light deteriorated wood actually darkens. This is due to the lignin which is responsible for the darkening and yellowing in wood degradation (Hallet and Bradley, 2003). Lignin is a photosensitizing organic polymer which can absorb radiant energy and transfer it to cellulose which initiates a cycle of degradation reactions though bond scissions. Additionally, the degradation products of lignin are both acidic and chromophoric which only exacerbates the yellowing effect (Hallet and Bradley, 2003). This is exemplified by the blank unpigmented wood coupon which exhibited a large ΔE* of 8.38 (graph 2), becoming darker and shifting towards yellow and green. The darkening of the wood is also clearly visually evident (fig. 15). This darkening and yellowing of the wood substrate are important to keep in mind when interpreting the color shifts in pigmented coupons, as the underlying color of the substrate may be a contributing factor. The unpigmented aged paper coupon did have a just noticeable ΔE* of 2.21. Pure paper is comprised of cellulose which is generally light stable. Impurities in paper such as lignin, however, can induce degradation when exposed to radiation. (Hallet and Bradley, 2003).

Of all the tested pigments and substrate combinations, purple yielded the largest ΔE* of 16.78 on paper and 14.88 on wood. Visually, the coupons do appear to have had a drastic color shift in addition to excessive fading. The purple pigmented coupons appeared quite dark and gray before aging. This is confirmed by the a* and b* values being close to 0 in the color sphere. Interestingly, the paper coupons appeared more purple after aging. As discussed in observations, the purple component to the pigment appeared to diffuse to the edges of tidelines. It is speculated that some pigments may have a stronger affinity with DCM than wood and hence the binding of the pigment only occurred where the DCM evaporated (Pittus, et al., 2018). It could be the case that the purple components diffused deeper into the substrate so that when the top surface faded, the purple components below became more evident. The wood coupons had just as drastic of a

color shift. The almost complete fading of the purple revealed much of the yellow wood substrate below. This drastic jump from showing no saturation to high saturation are likely the leading causes for the high ΔE* values in purple paper and wood coupons.

The ΔE* values for blue and yellow coupons are slightly misleading due to their weak pigmenting power. Although multiple coatings of pigment were applied during the preparation of the fungal coupons, a deeper level of blue saturation couldn't be achieved in the same way the purple and the red pigments achieved. Additionally, there was a very quick noticeable fading of the pigment in the span of an hour. The low saturation capacity of the blue pigment could be due to the composition of the xylindein molecule. Xylindein has an amorphous structure when dry which can increase the difficulty to interact with active bonding sites. Even more, xylindein has a strong affinity for hydrogen bonding due to the available hydroxyl (–OH) sections in the molecule and can tend to bind to itself (Pittus, et al.,2018). Since cellulose is a polymer with polar -OH bonding sites, there might be a slight repellence between the slightly polar cellulose polymer and the nonpolar amorphous xylindein molecule. The poor adherence of the blue pigment on paper and wood substrates may account for the low color stability observed.

The higher ΔE* for blue wood (12.1) than blue paper (8.98) is likely due to the same reason mentioned for the purple colorant in that once the blue faded, the darker yellow color of the substrate became more visible and contributed to the shifts in CIELab values. The same can be said about yellow paper and wood coupons. Yellow coupons are statistically the second most lightfast pigment of all the pigments tested with a ΔE* of 5.95 for paper and 6.74 for wood. This speaks to a caveat when using ΔE* to quantify lightfastness in that it can only measure the difference between color values. Thus, a coupon with low original saturation would have less overall color to lose and therefore report a smaller ΔE* value. In the case of yellow coupons, the

color of the wood substrate is in the 'yellow family' and is closer to the color of the overlying pigment. This likely explains the statistically low ΔE* for yellow coupons and higher ΔE* for blue coupons. Although both visually appear to have similar levels of fading post-aging, and both had lower saturation levels to start due to low pigmenting capacity of the pigments, the color bleed-through of the underlying wood contributed to very different shifts in CIELab values and ultimately, the resulting ΔE* measure of lightfastness. As the molecular structure of the yellow and purple pigments extracted from *S. ganodermophthorum* have yet to be identified, it is difficult to ascertain additional reasons for their weak pigmenting capacity and poor lightfastness.

The most lightfast of all the fungal pigments, both by visual indications and statistical ΔE* value, were the red coupons. Red paper had the lowest ΔE* of 2.87 which just crosses the threshold for color shifts perceivable by the human eye. If the slightly misleading low ΔE* from yellow coupons are disregarded, red wood was the next most lightfast coupon with a ΔE* of 6.94. The reported crystalline structure of the red naphthoquinone pigment may be contributing to its higher color stability. As aromatic conjugated compounds, naphthoquinones also have a higher ability to both delocalize energy through double bonds and to absorb and re-emit UV radiation at less energetic wavelengths in the form of heat or light (Gabros, et al, 2020). Additionally, the many substitution sites and the electronegativity of functional groups in the molecular structures of naphthoquinones allows for flexible bonding capabilities to both polar and nonpolar materials. Quinones could also adhere to cellulose fiber structures through hydrogen bonds formed between the hydrogen or oxygen atoms of the dye and the oxygen and hydrogen atoms of the cellulose -OH groups. Naphthoquinones are also larger molecules that can get more easily entrapped within the complex cellulosic structures (Dulo, et al, 2021). As the

results of this experiment indicate, pigments with crystalline structures offer superior light stability than those with non-crystalline structures.

6. Further Work

Future experimentation can expand upon the findings learned from this research by altering the parameters utilized in accelerated aging. The next round of accelerated aging should assume fungal pigments to be highly sensitive materials which, in simulating appropriate museum conditions, means the lux should be brought down to 50 lux per hour for a total of 145,600 lux hours per calendar year with UV filtration. Additionally, the pigments should be checked every hour to more precisely determine when the onset of just noticeable fading begins. This would enable better comparisons to how lightfast these pigments truly are relative to ISO BWS. It would also be beneficial, if possible, to explore methods to calibrate color measurements of wood coupons so that the color of the substrate can be subtracted or deconvoluted from the CIELab measurements.

Lastly, should fungal pigments find use in museum contexts, a greener alternative to DCM should be explored. DCM is a highly toxic solvent and contributes to greenhouse gas emissions. The requirement to solubilize fungal pigments in DCM, therefore, limits the accessible and sustainable use of these materials. Research into alternative solvent carriers was begun but fell out of the scope of this study. Future work can continue experimentation with alternative pigment carriers.

7. Conclusion

Spalting fungi are those which release pigments to produce unique and beautifully colored patterns in wood. Because the pigments and zone lines are released for competition and resource capture of nutrients, spalting tends to be a response to stress, whether from other fungi or poor environmental conditions (Robinson, 2019). For these reasons, the pigments secreted by fungi were reported to be incredibly stable with lightfast properties. The ability to quickly extract fungal pigments in specific colors, a process developed by Dr. Seri Robinson, has fueled advanced study, analysis, and experimentation of the material. In the literature, extract fungal pigments are purported to be very light stable. If true, this material would have promising applications to the field of conservation such as in the development of lightfast inpainting and coating materials. To date, however, the lightfastness of extracted fungal pigments has never been specifically quantified.

To determine whether these materials are lightfast by museum standard definitions, an accelerated aging experiment was conducted on fungal pigments applied to paper and wood coupons. After 24 hours of aging, the pigments exhibited high degrees of fading. Although an ISO BWS card was aged alongside the museum and faded through to BWS #5, the pigments had faded too much to make a direct comparison to a specific BWS target. Of all the pigments, the red produced by *S. cuboideum* proved to be the most lightfast. Its superior resistance to fading is likely due to the stability afforded by the crystalline structure of the naphthoquinone molecule. Although extracted fungal pigments did not prove to be more light stable than other natural dyes and colorants, they are a fascinating material to continue exploring and experimenting with.

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