Effect of Ozonation on Fungal Resistance of Bamboo and Oak Flooring Materials

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Abstract

Lignocellulosic materials are gaining increased interest as renewable sources of building materials. However, chemical and microbiological degradation can occur when lignocellulosic materials are exposed to environmental stressors such as ozone and elevated humidity. In this study, the effects of ozone treatment and solvent extraction on fungal growth rates of bamboo and oak flooring materials were investigated. One set of samples was extracted with a mixture of cyclohexane and ethanol solvents for 72 h to remove extractable compounds. Another set of materials was exposed continuously to ozone (2000 $\mu L.m^{-3}$ or 2000 ppb_v) for one to five weeks. Solvent-extracted and ozone-treated samples were incubated in closed chambers at 85 % or 55 % RH and 30 ^oC. Incubated samples were removed at regular time intervals for fungal growth evaluation. Ozone treatment caused chemical changes in bamboo and oak, which appeared to reduce bamboo's resistance to fungal attack. Longer ozone exposure led to higher susceptibility to fungal growth. Untreated and ozone-treated oak showed no evidence of fungal growth, suggesting that this material may contain fungi-inhibitory compounds that are not removed by these treatments. Also, a delay in fungal growth on cyclohexane/ethanol-extracted bamboo was observed, probably due to the extraction process removing substances that enhanced fungal growth.

Research Highlight

- We evaluated the effects of ozone exposure and cyclohexane/ethanol extraction on fungal resistance of oak and bamboo;
- Ozone treatment appeared to reduce bamboo's resistance to fungal attack
- Cyclohexane/ethanol extraction appeared to result in a delay in fungal growth on bamboo, probably due to substances that enhanced fungal growth removed by the extraction process
- No effect were observed for oak materials

Keywords

Fungi, Ozone, Extractives, Flooring, Oak, Bamboo

1. Introduction

Cellulose-based materials have been used for centuries for many functions such as framing, furniture and flooring in both residential and commercial construction. Traditional materials for interior applications include oak, pine and maple. Recent emphasis placed on the use of renewable building materials have led to increased interest in fast growth, abundant lignocellulosic species such as bamboo for interior applications [Lugt et al. 2006]. However, the susceptibility of cellulose-based building materials to mold growth is a potential concern. Research has shown that mold growth on various products, e.g., plasterboards, cellulose insulation, cellulose-containing ceiling tiles, particleboards, and cellulose-based flooring materials can be significant [Peitzsch 2012, Dillavou et al. 2007, Herrera 2005,, Karunasena et al. 2000]. Recent studies by Hoang et al. [2010] and Johansson et al. [2013] indicated that cellulose-rich materials are highly susceptible to mold growth when they are exposed to liquid water or high relative humidity (RH). Different cellulose-based materials support mold growth at

different levels, perhaps due in part to some species containing natural antifungal compounds that prevent or minimize fungal growth. The principal components of lignocellulosic material are high molecular masses of lignin and carbohydrates and a small amount of low molecular mass extractives [MacDonald et al. 1969]. Although extractives make up only minor, nonstructural components, they are often important in contributing to many material characteristics for interior uses, such as odor, color, wettability, permeability, and resistance to decay and insects [Hse and Kuo, 1988]. The effects of extractives on fungal growth have been studied, and different extracted compounds appear to be inhibitory to selected fungal species [Zhang et al. 2010, Min et al. 2008].

Ozone is a powerful oxidizing agent and readily reacts with unsaturated organic compounds [Al Mulla et al. 2010, Weschler 2000, Bailey 1982]. Ozone has an oxidation potential of 2.07 V at 25 ^{o}C, and a viable disinfectant in both aqueous and gaseous phase [EPA 1999, Kim et al. 1999]. It has been used as an alternative disinfectant and reactant in several processes such as wastewater treatment, odor elimination, and pesticide removal. Because it possesses bactericidal properties and deactivates fungal spores, ozone has also been used to disinfect buildings [Poppendieck et al. 2007, Currier et al. 2001; Palou et al. 2003]. Dyas et al. [1983] showed that ozone concentrations as low as 0.3 $\mu L \cdot L^{-1}$ to 0.9 $\mu L \cdot L^{-1}$ (0.3 ppm_v to 0.9 ppm_v) had useful bactericidal action against human pathogens. Taylor and Morrell [2009] reported that ozone appeared to deactivate fungi on cellulose-based surfaces. Foarde and Eaton [2007] observed that the biocidal efficacy of ozone against selected organisms deposited on a glass slide was higher than against the same organisms on a gypsum wallboard, partly because gypsum reacts with ozone and protects the spores. Unfortunately, because of its high oxidizing potential, ozone has been identified as a common indoor pollutant at relatively low concentrations, from less than 5 $\mu L \cdot m^{-3}$ (5 ppb_v) to 50 $\mu L \cdot m^{-3}$ (50 ppb_v) [Weschler 2000].

The effects of ozone reactions with building materials have been reported. For example, exposure to ozone has been found to cause chemical degradation and generate secondary products from cellulose-based materials [Hoang et al. 2009, Poppendieck et al. 2007, Nicolas et al. 2007, Lemeune et al. 2003, Morrison and Nazaroff 2002]. Poppendieck et al. [2007] studied the reaction of 24 building materials with ozone at high concentrations and observed elevated releases of reaction products from many materials. Ozone is commonly used for delignification of lignocellulosic products in the paper industry. In addition, ozone can oxidize other components, such as cellulose and hemicelluloses [Kobayashi et al. 2005, Lyse 1979, Byrd 1992]. As such, ozone can change the chemical composition of cellulose-based materials, a process that might alter the susceptibility of materials to fungal growth. For example, terpenoids in oak are believed to protect this material from fungal attack as well as from ozone damage [Leudau, 2007]. These compounds are highly reactive with ozone and reactions with ozone lead to the formation of carbonyls and carboxylic acids (Norgaard et al. 2013, Forester and Wells 2011, Hoang et al. 2009, Poppendieck et al. 2007, Weschler 2000). Currently, little information exists with regard to the effects of ozone treatment on fungal resistance or surface chemistry of hardwoods (e.g. oak) or bamboo commonly used for flooring.

The main objective of this study was to assess the influence of ozone exposure on fungal resistance, surface chemistry, and water sorption of bamboo and oak flooring materials. These properties, i.e., surface chemistry, moisture content, and type and amount of extractives, all play an important role in fungal resistance. Another objective was to determine the effect of solvent extraction on the fungal growth of these two cellulose-based materials. An elevated ozone concentration of 2000 ppb was used in this study, consistent with the extensive oxidation

chemistry that may occur in buildings during fungal remediation or odour elimination. This rapid oxidative "aging" approach might also be considered as a screening assessment tool to identify materials for which longer-term oxidation at lower ozone concentrations may alter material susceptibility to fungal attack.

2. Experimental Methodology

Oak and bamboo flooring materials were chosen to evaluate the effects of ozone treatment or solvent extraction on fungal resistance. Each material was purchased from a home improvement store. Upon purchase, the materials were wrapped in multiple layers of plastic sheeting before the experiments. Both oak and bamboo flooring materials were prefinished products that had a polymeric coating on one side, referred herein as the front side. The back side of each test specimen was polished with sand paper (Super fine 400 Grit) prior to conducting the experiments. The materials were cut to identical specimen sizes of 2.5 cm x 2.5 cm x 0.4 cm for oak and 2.5 cm x 2.5 cm x 1.1 cm for bamboo. The specimens were treated either with ozone or extracted with a mixed organic solvent as described below.

2.1 Ozone Treatment

Six samples of each selected material were placed in a 4-L glass flask (Figure 1) that was ventilated continuously with inlet air containing 2000 $\mu L.m^{-3}$ (2000 ppb) ± 63 $\mu L \bullet m^{-3}$ (63 ppb) of ozone for periods of one, three, or five weeks (equivalent to the ozone doses of 340 $\mu L \bullet L^{-1} \bullet h$, 1000 $\mu L \bullet L^{-1} \bullet h$, and 1600 $\mu L \bullet L^{-1} \bullet h$ respectively). Ozone was produced and monitored by a photometric ozone calibrator (Teledyne Instruments, M703E). Flow rate and lamp intensity were adjusted to deliver the specified ozone concentration. Since the air change rate of the chamber was high (45 h^{-1} to 75 h^{-1}), the concentrations of ozone in the flask and at the flask exhaust inlet were similar. The relatively high concentrations of ozone used in experiments were intended to accelerate the effects of surface chemistry and fungal resistance. Chemical changes at or near the material surfaces that were induced by the ozone treatment were measured by Fourier transform infrared (FTIR) spectroscopy in the attenuated total reflection (ATR-FTIR) mode. FTIR spectra were recorded at a 4 cm^{-1} resolution using dry air as a purge gas and a spectrometer (Nexus 670, Thermo Nicolet) equipped with a liquid nitrogen-cooled mercury cadmium telluride (MCT) detector. A ZnSe prism was used for the ATR-FTIR measurement. All spectra were the average of 128 scans. The peak height was used to represent the infrared intensity, which is expressed as absorbance (A).

2.2. Solvent Extraction

Another set of specimens (triplicate samples of each selected material) was extracted with a mixture of cyclohexane and ethanol (cyclohexane/ethanol) in a soxhlet extractor. The extraction procedure was adjusted from ASTM D1105-96 [2007], with a slight modification of the solvent content (cyclohexane: ethanol = 2:1 by volume). The extraction was carried out for 72 hours with approximately 12 cycles (siphonings) per day. When the extraction was completed, extracted specimens were preserved for fungal resistance testing. The extractive-containing solvent remaining in the flask was dried to a constant mass for determining the percentage of the extract removed from the lignocellulosic materials. Component groups in extracts were analyzed by thermal desorption gas chromatography/mass spectrometry (GC/MS).

2.3. Sorption Isotherms

To better understand the relationship between fungal growth and moisture content of the two selected materials used in this study, water vapor sorption isotherms of bamboo and oak were obtained using a moisture sorption analyzer (IGASORP, Hiden Analytical). This instrument is equipped with a microbalance that can provide a mass resolution of 10^{-7} g. All sorption measurements were carried out at 30 ^{o}C using approximately 20 mg samples, which were carved out from near the surface of the 2.5 cm x 2.5 cm specimens. The results of two or three specimens were averaged and variations between specimens reported as the range.

2.4. Fungal Resistance Assessment

Ozone treated specimens were incubated in temperature and humidity controlled chambers at 30 ^{o}C ± 1 °C and 85 % ± 1 % RH and at 30 °C ± 1 °C and 55 % ± 1 % RH. Solvent extracted specimens were only incubated in conditioning chambers at 30 ^{o}C ± 1 °C and 85 % ± 1 % RH. Fungal growth of untreated (control) specimens was also investigated for comparison. All specimens were monitored periodically over a period of five months with an optical microscope to detect fungal growth on material surfaces. This experiment was intended for evaluation of the resistance of common fungi on bamboo and oak surfaces. No specific fungal species was introduced to inoculate the exposed specimens. Instead, natural inoculation from species in the environment was allowed to occur during the incubation period. Temperature and humidity controlled lab air was introduced continuously to the incubation chambers. The aforementioned experiments were conducted with all tested specimens at the same time to ensure the same exposure and incubation conditions for the purpose of comparison.

3. Results

3.1. Effect of Ozonation on Moisture Sorption of Bamboo and Oak

The ability of fungi to grow on lignocellulosic materials depends strongly on the moisture content (MC) of the cell wall. Fungi can only degrade cellulose-based material when free (liquid) water is present, which occurs only after cell walls are saturated with water [Rowell 2005]. Further, the MC has a strong influence on the reactions between ozone and lignin compounds in wood [Mamleeva et al. 2008]. Therefore, knowledge of the relationship between RH and MC in the red oak and the bamboo materials used in this study is important for understanding RH conditions that promote the growth of fungi on these materials before and after ozone treatment. The amount of water uptake in cell walls is a combination of adsorption with three major chemical constituents, namely, hemicellulose, cellulose, and lignin. Among the three, hemicellulose adsorbs the most water and lignin the least [Rowell 2005]. Figure 2 presents the relationship between RH and equilibrium MC (i.e., moisture sorption isotherms) of untreated and ozone-treated oak and bamboo measured at 30 ^{o}C and atmospheric pressure. The bars show standard deviations, which indicate little difference in values at the same RH between specimens. These moisture sorption isotherms are used to estimate the MC in red oak and bamboo at a particular RH. It is noted that the moisture content obtained by the isotherm experiment in this study is expressed as the mass of water uptake with respect to the mass of sample specimens that were dried at approximately 0 % RH at 30 ^{o}C for 24 h. Both materials had a similar sorption behavior as a function of RH, though the oak took up slightly more water than bamboo in the 5 % to 90 % RH range (Figure 2). The amounts of water sorbed to these two materials were different for the two incubation humidity levels used in this study. At 55 % RH, oak took up approximately 7.5 % water while bamboo adsorbed about 6.5 %. However, at 85 % RH, the two

materials took up essentially the same amount of water. It is worthwhile to note here that the term adsorption is correct for describing the water uptake at 55 % RH, but is incorrect for that at 85 % RH or higher. This is because at very high humidity (>75 % RH), both adsorption (a phenomenon that causes swelling and produces heat) and absorption (a phenomenon occurring only in the pre-existing pores that does not cause swelling and does not produce heat) processes take place [Chin et al. 1999]. In the humidity range from 10 % to 70 %, the slope of the isotherm was relatively low, approximately 1 % MC increase for every 10 % increase in RH (as shown in Figure 2A). In this MC range, only bound water adsorbed strongly (by hydrogen bonding) to cell walls. Fungi are not expected to grow on these two materials in this MC range. However, when the surrounding RH reaches 75 % or higher, the MC of both bamboo and oak increased sharply, indicating the presence of both bound water and liquid water in the capillaries of the material microstructure. Fungi will likely grow in this high RH range.

The effect of ozone treatment on the water sorption isotherms of these selected materials can be seen in Figure 2. Ozone treatment appeared to increase slightly the water sorption of oak in the 40 % to 85 % RH range, but had little effect on the water uptake of bamboo in the same RH range. The slight increase in moisture sorption for oak may be explained by the formation of acid and aldehyde groups during ozonation. As discussed in Section 3.3, an increase in intensity of the C=O group (assigned at the absorption band of 1730 cm^{-1} as shown in Figure 4A) was due to the oxidation of the side chains of lignin molecules, and a small fraction was from oxidation of hemicellulose. This group is highly hygroscopic and have a strong affinity for water. However, the lack of a noticeable increase of water uptake following ozone treatment of bamboo is surprising, considering the fact that an increase in intensity of the C=O group in this material was also observed.

3.2 Extractive Components of Bamboo and Oak

The cyclohexane/ethanol extracts made up approximately 3.4 % and 2.6 % of dried material mass for oak and bamboo, respectively. These percentages would likely have been higher since some volatile compounds are assumed to have evaporated during the drying of the extracted solution. More than thirty volatile and semi-volatile compounds were identified in the extracts of bamboo and oak (Table 1). Seventeen compounds were common to both materials, but more than half are different. It is notable that the extraction with a mixture of apolar cyclohexane and low polar ethanol at a volume of 2:1 ratio is expected to remove mostly apolar and low-polar compounds in the extractives, but is unlikely to remove significant amounts of the polar and highly polar extractive compounds.

3.3. Effects of Ozonation on Chemical Changes of Bamboo and Oak Surfaces

Ozone has been known not only to preferentially attack lignin but also oxidize the carbohydrate constituents in wood [Lyse 1979, Mamleeva et al. 2009, Byrd et al. 1992, Ragnar et al. 1999]. Therefore, some information on chemical constituents and their distributions in wood is provided here to better understand the effect of ozone treatment on bamboo and oak. The main components of wood are cellulose (40 % to 50 %), lignin (15 % to 35 %), hemicellulose (20 % to 35 %), and solvent-soluble extractives (3 % to 10 %) such as terpenes, tannins, aromatic and aliphatic acids [MacDonald et al. 1969]. About 70 % of lignin is located between the fibers, and lignin of hardwoods (e.g., oak) tends to concentrate in the outer walls of fibers. Hemicellulose and lignin are amorphous polymers, while cellulose is a crystalline polymer for which ozone only attacks its amorphous regions [Lyse 1979, Byrd et al. 1992]. ATR-FTIR spectra (in the

region of 3800 cm^{-1} and 800 cm^{-1} of untreated bamboo and oak are presented in Figure 3. It should be noted that with a refractive index of 1.43 for wood [Zavarin et al. 1991], the probing depth by the ATR technique in wood materials was between 0.13 μm and 2.15 μm from the surface. This probing depth is appropriate for the present study, because the effect of ozone treatment on wood and other reactive materials only occurs at or near the surface [Schuerch 1963, Byrd et al. 1992]. However, both spectra in Figure 3 show an overlap and similarity of a number of absorption bands that represent different chemical groups in the examined materials. The basic structure of lignocellulosic materials was observed with a strong, broad hydrogen-bonded OH stretching between 3330 cm^{-1} and 3800 cm^{-1} and CH stretching of methyl and methylene groups in the 2750 $cm^{-1}$to 3000 cm^{-1} region [Shigeru et al. 2005]. The band centered at around 1510 cm^{-1} is due to the aromatic stretching vibrations of the lignin component [Owen and Thomas 1989, Colom et al. 2003]. The absorbance at 1730 cm^{-1} is characteristic of the C=O stretching vibration of the holocellulose components (i.e., cellulose and hemicellulose) [Owen and Thomas 1989, Bodîrlău and Teacă 2009]. The bands centered at 1020 cm^{-1} and 1230 cm^{-1} were assigned to the aliphatic and aromatic C-O stretchings, respectively [Colthup et al., 1990].

The influence of ozonation on chemical properties of oak and bamboo is presented in Figure 4, which shows changes in intensities of the 1510 cm^{-1} and 1730 cm^{-1} bands as a function of ozone exposure. The intensities were normalized to that of the 895 cm^{-1} band, which had been observed essentially unchanged with ozone treatment [Muller et al. 2003]. Although the standard deviations for each type of sample were relatively large, ozonation appeared to cause an increase in intensity of the band at 1730 cm^{-1} in both oak and bamboo and a decrease in intensity of the 1510 cm^{-1} band in bamboo. The observation of an increase in intensity of the band at 1730 cm^{-1} was similar to that reported by Kobayashi et al. [2005] for ozone treatment in the liquid phase of white birch and Japanese cedar. However, these authors did not observe the appearance of the 1730 cm^{-1} band in these two materials treated with ozone in the gas phase. Based on evidence of FTIR analysis of ozone treatment of cellulose, Kobayashi et al. [2005] suggested that the increased intensity at 1730 cm^{-1} was due to carbonyl C=O produced by cleavage of the glycosidic linkage of the cellulose. However, based on an intensive study of gas-phase ozonation of fiberized wood, Lyse [1979] concluded that the majority of the 1730 cm^{-1} band, which was assigned to non-conjugated C=O of acids and aldehydes, was due to the oxidation of the side chains of lignin molecules, and a small fraction was from oxidation of hemicellulose. Regardless of the mechanisms, the intensity increase at 1730 cm^{-1} indicated that the ozone treatment conditions employed in this study had oxidized the red oak and bamboo. However, ozone treatment did not appear to affect the 1510 cm^{-1} band (from lignin) of red oak, but caused a reduction of this band for bamboo.

3.4. Fungal Resistance of Ozone Treated and Solvent Extracted Oak and Bamboo

Figure 5 presents the time when the mold was first observed on both untreated and treated bamboo specimens incubated at 85 % RH and 30 ^{o}C versus the ozone exposure dose. Exposure, in $\mu L.L^{-1}.h$ units is defined here as the concentration of ozone that passed over a specimen surface integrated over the exposure time. Fungi were first observed to grow at approximately Week 10 for specimens exposed to an ozone dose of 340 $\mu L \bullet L^{-1} \bullet h$ (i.e., one week of ozone treatment). This time-to-mold-growth was slightly shorter than that for the controls (untreated bamboo). For higher dose treatments, the times it took for fungi to appear and spread were shorter. For example, it took only six weeks and one month for fungi to start growth on bamboo exposed to 1000 $\mu L \bullet L^{-1} \bullet h$ and 1600 $\mu L \bullet L^{-1} \bullet h$ ozone exposure, respectively. These results

indicate that the higher the ozone dose, the shorter the time it took for fungi to grow on bamboo incubated at 85 % RH and 30^{o}C condition. A similar trend was observed for the times when the surface was completely covered with fungi, as shown in Figure 6. This figure displays representative optical microscopy images taken after the fungi have spread across the entire surface of untreated bamboo and bamboo treated with ozone for different lengths of time (i.e., different ozone doses). It is clear that longer ozone exposures led to a faster spread of fungi on the bamboo. For example, it took less than four days for fungi to cover the entire surface of specimens treated with ozone for five weeks, but it took nearly five times longer for fungi to achieve the same coverage on the control specimens. Fungi were not observed on untreated or ozone-treated bamboo specimens exposed to 55 % RH at 30 ^{o}C. Also, no evidence of fungal growth was observed on untreated or ozone-treated oak specimens exposed to 85 % or 55 % RH (data not shown).

Solvent extraction appeared to increase the fungal resistance of bamboo. As compared to control specimens, solvent-extracted bamboo samples showed a two-week delay in fungal spore germination. No fungi were observed on extracted oak samples (data not shown). This finding was supported by previous studies. Hosseinaei et al. (2012) performed liquid hot water extraction on southern yellow pine flakes under different temperatures and the extraction of extraction of hemicelluloses resulted in improvement in fungal resistance of the test specimens.

4. Discussion

Ozonated bamboo specimens yielded more rapid fungal germination and produced more visible fungal growth than untreated specimens under the same experimental conditions. Longer ozone exposure, e.g., 1600 $\mu L \cdot L^{-1} \cdot h$, caused bamboo to be more susceptible to fungal growth, shortening the germination period by almost two months (Figure 5). These findings suggest that elevated ozone exposure may appear to break down some material components that inhibit, or/and produce compounds that are more favorable to, fungal growth. For instance, lignin is generally considered highly resistant to fungi [Schwarze 2007, Dekker et al. 2002]. Loss of lignin and formation of carbonyl groups during ozonation of bamboo appear to have occurred based on FTIR analysis (Figure 4). The intensity of the lignin band of bamboo at 1510 cm^{-1} decreased by 25 % after five weeks of exposure to ozone. This is consistent with previous ozonation studies of wood [Lyse 1979, Byrd et al. 1992, Mamleeva et al. 2008]. The absence of a change in the 1510 cm^{-1} band of the oak lignin after ozone treatment cannot be explained at this time. One possible reason is that terpenoids and terpenes contained in oak may react with ozone to avoid ozone damage to lignin and to provide additional protection from fungal attack [Lerdau 2007, Saddiq and Khayyat, 2010]. Another reason may be the effect of ozone treatment on the high density oak (density = 0.74 cm^3/g vs. 0.3 g/cm^3 for bamboo) was only on the top surface layer, which was not distinguished from the large sampling depth by the ATR-FTIR technique. Using the 45° angle and refractive indices of 1.43 and 2.2 for lignocellulosic material and the ATR crystal, respectively, the penetration depth of the evanescent wave (generated by the ATR technique) in lignocellulosic material was estimated to be 1850 nm at the 1510 cm^{-1} band. If the ozone-affected layer was only a few nanometers on the surface, its ATR-FTIR intensity was not likely to be separated from this large sampling depth.

Increases in the band centered at 1730 cm^{-1} indicated formation of new carbonyl groups on both bamboo and oak. Similar findings were reported for wood irradiation by UV light [Muller et al. 2003, Tolvaj et al. 2011], exposure to artificial light [Rosu et al. 2010], exposure to gas-phase ozone [Mamleeva et al. 2008], and exposure to liquid phase ozone [Kobayashi et al.

2005]. The higher fungal susceptibility of ozone-treated specimens may be explained by the formation of the hygroscopic C=O groups on the material surface. These groups could provide a favorable source for fungi to grow or good sites for water to form multiple monolayers, which have the effect mentioned earlier. This hypothesis requires further study before specific mechanisms for enhanced fungal growth are identified. No fungi were observed on red oak samples, including ozone treated specimens. This observation suggests that oak may contain some inhibitory compounds that prevent fungal growth. Some compounds in the cyclohexane/ethanol extract for oak had been identified as having antifungal effects. Inhibitory effects of benzaldehydes, vanillin, or phenol, for example, were observed on the growth of various fungal species [Fitzgenald et al. 2005, Kim 2011, López-Malo 1995]. It is notable that with the limit of GC/MS retention time to 14 minutes, many other compounds that may be extracted by cyclohexane/ethanol solvent were not identified in this study. In addition, the amount of extractives and their components also varied according to botanical species and/or the solvent used for the extraction, for example by dichloromethane [Vichi et al. 2007], ethanol/water [Caldeira et al. 2006], or hot water [Velmurugan et al. 2009]. A number of studies confirmed the antimicrobial activity of oak extract based on phenolic compounds [Velmurugan et al. 2009, Cadahía et al. 2001] and tannins [Min et al. 2008]. Monoterpenes emitted from some oak species [Kesselmeie et al. 1996] were also observed to inhibit the growth of bacteria and fungi [Saddiq and Khayyat 2010]. It is possible that oak contains sufficient amounts of terpenoids to avoid meaningful depletion by ozone. Bamboo, however, appears to be rendered more susceptible to fungal growth following terpenoids-depletion.

Bamboo, is well known as being susceptible to mildew due to its abundant starch and sugars [Li 2004, Zhang et al. 2010]. In addition, considerably less growth was observed on solvent extracted bamboo specimens, and it took longer for fungi to start germinating on these samples. It is possible that the extraction removed some chemical components that encourage the growth of fungi on bamboo. Zhang et al. [2010] reported similar observations, which indicate that some bamboo species contain extractives that promote bamboo biomass infestation by fungi. However, no specific compounds were identified by Zhang et al. [2010]. Some compounds extracted from bamboo, for example hydroquinone (presented in Table 1), are reported as the carbon source for some fungal species [Harbison and Belly 2009].

Although information on the chemical identity of extractives for both bamboo and oak as given in Table 1 is important, further systematic research is needed to assess the role of each of these components, and its interaction with ozone, on the fungal activity of bamboo and wood flooring alternative materials. In our study the untreated oak and bamboo surfaces were freshly sanded prior ozonation. However, extractives in these two materials tend to migrate to their surface with time in service [Hse and Kuo, 1988]. The effect of this migration during the service life of these materials on fungal growth is unknown. Such information is needed to more effectively assess the suitability of ozonation as a viable method for building disinfection and, perhaps, to better understand the role of oxidative aging on material susceptibility to fungal growth

Humidity plays a key role in fungal growth on bamboo, as fungi were only observed in the case of 85 % RH. This finding is in agreement with previous studies that found no evidence of fungal growth on a number of materials at RH of 70 % or lower [i.e., Pasanen et al. 2000, Nevalainen and Seuri 2005]. Hoang et al [2010] also observed that fungal spores are only germinated after the moisture content of building materials reaches a threshold. Figure 1 shows a steep water uptake by both bamboo and oak when the RH reaches 75 % or higher, after which

water exists in both adsorbed and free forms. The presence of liquid water is believed to promote fungal growth on lignocellulosic materials. In addition, both cellulose and hemicellulose in bamboo and oak contain abundant hydroxyl groups that can readily adsorb water [Xie et al. 2011]. At very high humidity (> 85 %), multiple water layers are formed on the hydroxyl groups of hemicellulose and cellulose's amorphous regions of the cell walls, in which case the outer monolayers exist in the form of liquid water. Therefore, liquid water in both the material structure capillaries and on the cell walls is believed to contribute to fungal attack at high RH.

5. Conclusions

Ozone exposure caused chemical transformation on both bamboo and oak flooring materials with new carbonyl group formation and lignin degradation. Although these chemical changes were likely responsible for reducing the fungal resistance of bamboo, the modification/removal of natural anti-fungal compounds (e.g., terpenoids) by ozone treatment may also contribute to the fungal susceptibility of bamboo. The longer bamboo was exposed to ozone, the more susceptible it was to fungal growth. This impact should be taken into account if elevated ozone is selected as a building disinfectant or odour elimination. Ozone exposure also slightly increased the moisture sorption capacity of oak but did not seem to affect water uptake by bamboo. This study has also identified the chemical components in the cyclohexane-ethanol soluble extractives of these two lignocellulosic materials; such components might play an important role in fungal growth and other properties of these materials. Additional research is needed on exposure of lower ozone concentration and a wider spectrum of extracted products and consideration of volatile compound losses during the extraction and evaporation procedures, and to explore how the intense but short-term ozone exposures.

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*** Certain commercial product or equipment is described in this paper in order to specify adequately the experimental procedure. In no case does such identification imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that it is necessarily the best available for the purpose.*

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Table 1. Cyclohexane/ethanol extracts of oak and bamboo

OAK		BAMBOO	
Retention time (minutes)	**Chemicals**	**Retention time (minutes)**	**Chemicals**
2.72	Hexanal	3.05	Furfural
3.05	Furfural	3.27	2-Furanmethanol
3.58	Cyclohexanol	3.58	Cyclohexanol
3.70	Cyclohexanone	3.71	Cyclopentanone, 2-methyl-
4.46	Benzaldehyde	4.46	Benzaldehyde
4.56	Hexanoic acid	4.56	Hexanoic acid
4.64	Phenol	4.73	Tetraethyl silicate
4.73	Tetraethyl silicate	4.87	Decane
4.87	Decane	5.19	1-Hexanol, 2-ethyl-
4.91	Octanal	5.76	2,5-Furandicarboxaldehyde
5.20	1-Hexanol, 2-ethyl-	6.05	Nonanal
5.90	Dodecane, 2,6,10-trimethyl-	6.55	Cyclopentasiloxane, decamethyl-
5.99	Octane, 2,3,6,7-tetramethyl-	6.98	Cyclododecane
6.02	Undecane, 3,8-dimethyl-	7.14	Decanal
6.05	Nonanal	7.24	Benzofuran, 2,3-dihydro-
6.55	Cyclopentasiloxane, decamethyl-	7.35	2-Furancarboxaldehyde, 5-(hydroxymethyl)-
6.69	Octanoic Acid	7.77	Hydroquinone
6.98	1-Dodecene	8.29	2-Methoxy-4-vinylphenol
7.14	Decanal	8.32	Cyclohexasiloxane, dodecamethyl-
7.25	Benzofuran, 2,3-dihydro-	8.67	Benzaldehyde, 4-hydroxy-
7.44	Benzothiazole	8.97	1-Tetradecene
7.89	Decane, 2,3,7-trimethyl-	9.12	Vanillin
8.06	cis-3-Methyl-4-octanolide	9.43	Ethanone, 1-(2-hydroxyphenyl)-
8.32	Cyclohexasiloxane, dodecamethyl-	10.09	Phenol, 2,4-bis(1,1-dimethylethyl)-
8.34	Heptacosane	10.15	Butylated Hydroxytoluene
8.39	2(3H)-Furanone, 5-butyldihydro-4-methyl-, cis-	10.32	10-Methylnonadecane
8.53	Hydroxylamine, O-decyl-	10.75	1-Nonadecene
8.68	Benzaldehyde, 4-hydroxy-	11.20	Benzophenone
8.97	Trichloroacetic acid, undecyl ester	11.40	Benzaldehyde, 4-hydroxy-3,5-dimethoxy-
9.12	Vanillin	12.00	4-Hydroxy-2-methoxycinnamaldehyde
10.09	Phenol, 2,4-bis(1,1-dimethylethyl)-	12.27	Benzoic acid, 4-hydroxy-3,5-dimethoxy-, hydrazide
10.15	Butylated hydroxytoluene	12.35	Dichloroacetic acid, heptadecyl ester
10.75	1-Docosene	12.68	Aspidinol
10.86	Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester	13.60	2-Ethylhexyl trans-4-methoxycinnamate
11.21	Benzophenone	13.82	3,5-Dimethoxy-4-hydroxycinnamaldehyde
11.40	Benzaldehyde, 4-hydroxy-3,5-dimethoxy-		
13.54	Tetradecanoic acid		

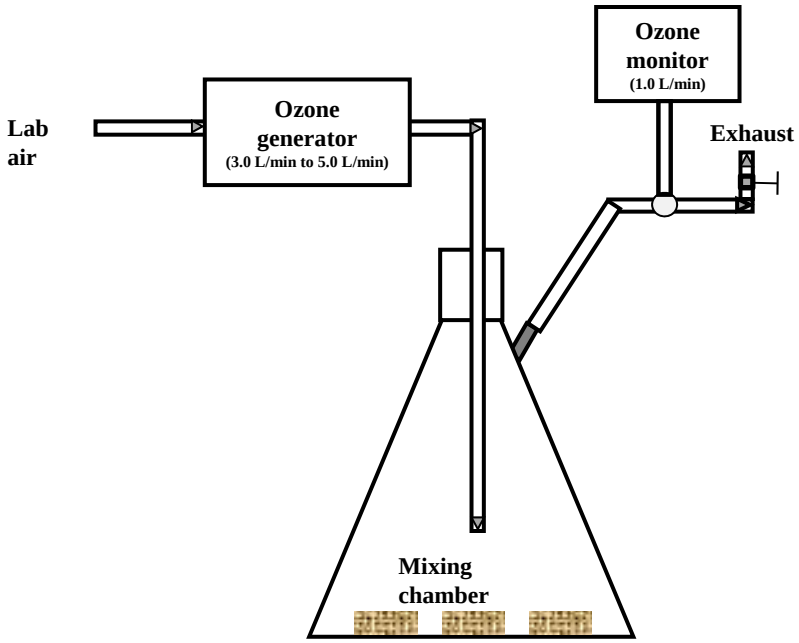

Figure 1. Experimental apparatus for ozone treatment of bamboo and oak samples.

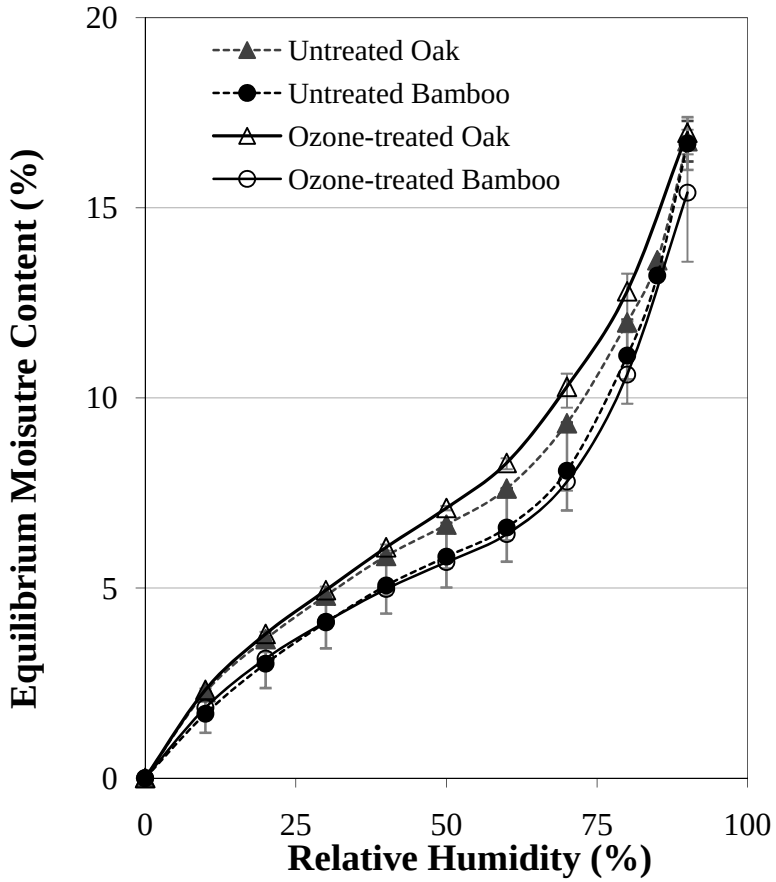

Figure 2. Moisture sorption isotherms of bamboo and oak before and after ozonation. Symbols are experimental data and lines are best fitting curves. Error bars represent value ranges

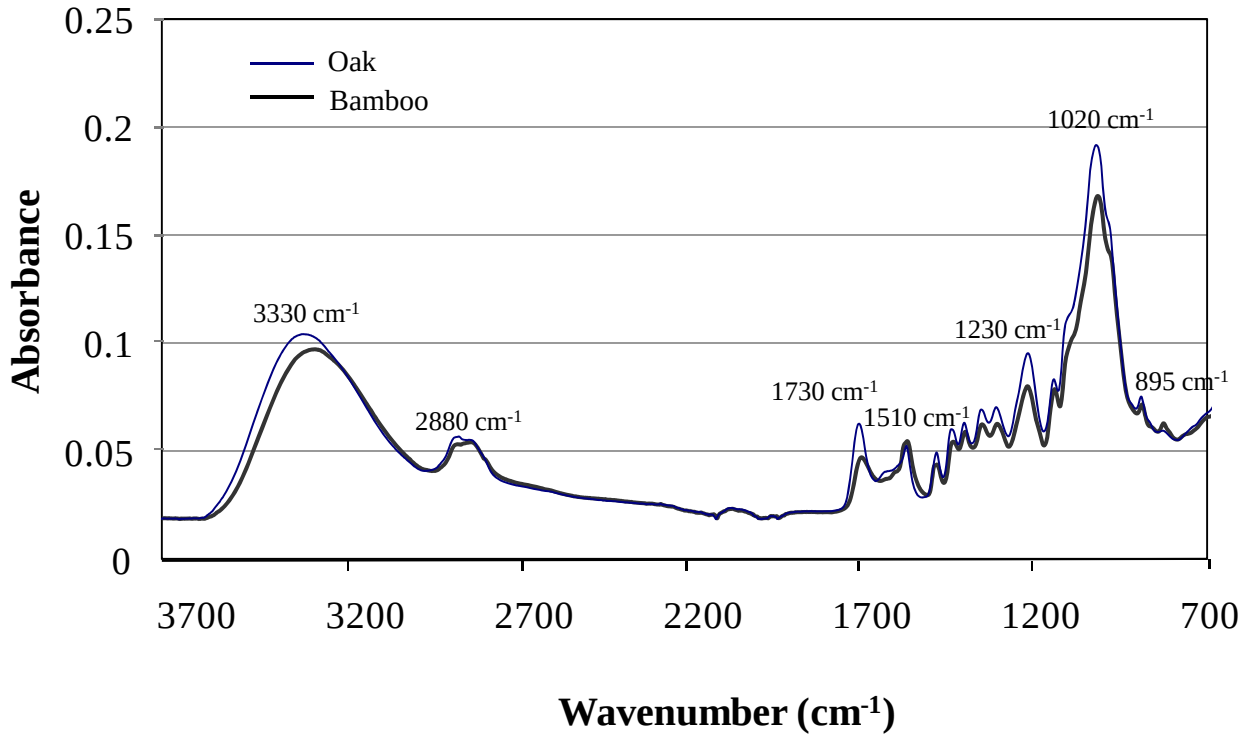

Figure 3. ATR-FTIR spectra of untreated bamboo and untreated oak.

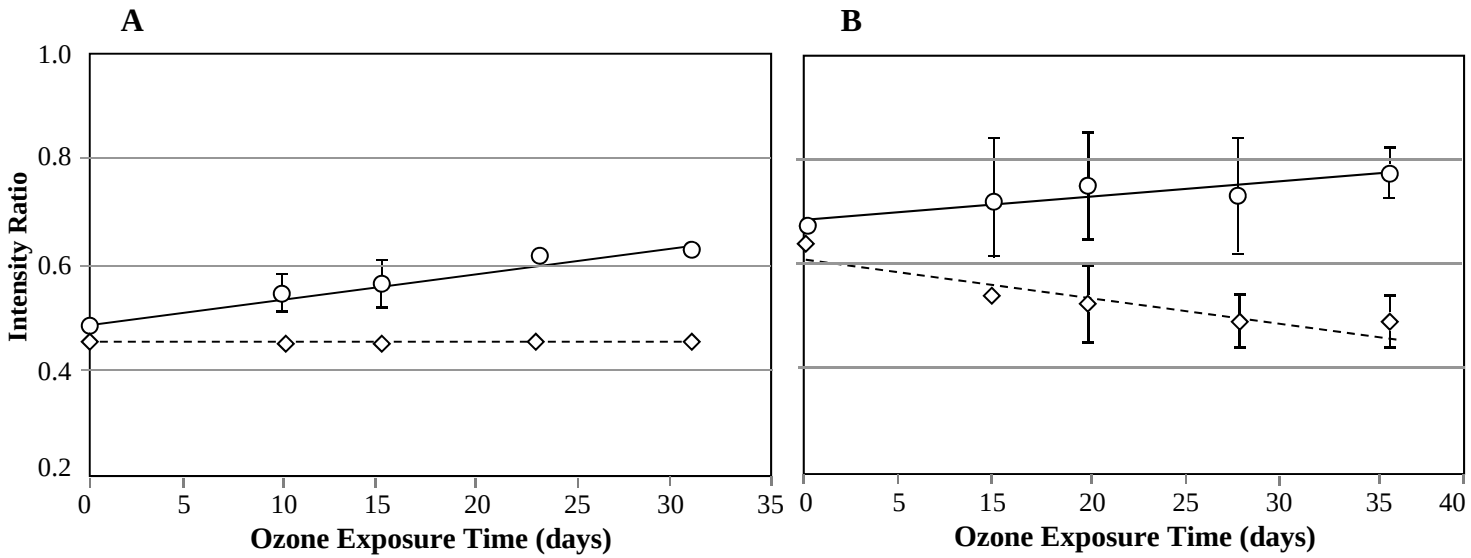

Figure 4. ATR-FTIR Intensity changes of the 1510 cm^{-1} (◊) and 1730 cm^{-1} (○) bands as a function of ozone exposure for oak (A) and bamboo (B). The intensities have been normalized to that of the 895 cm^{-1} band. The error bars represent one standard deviation.

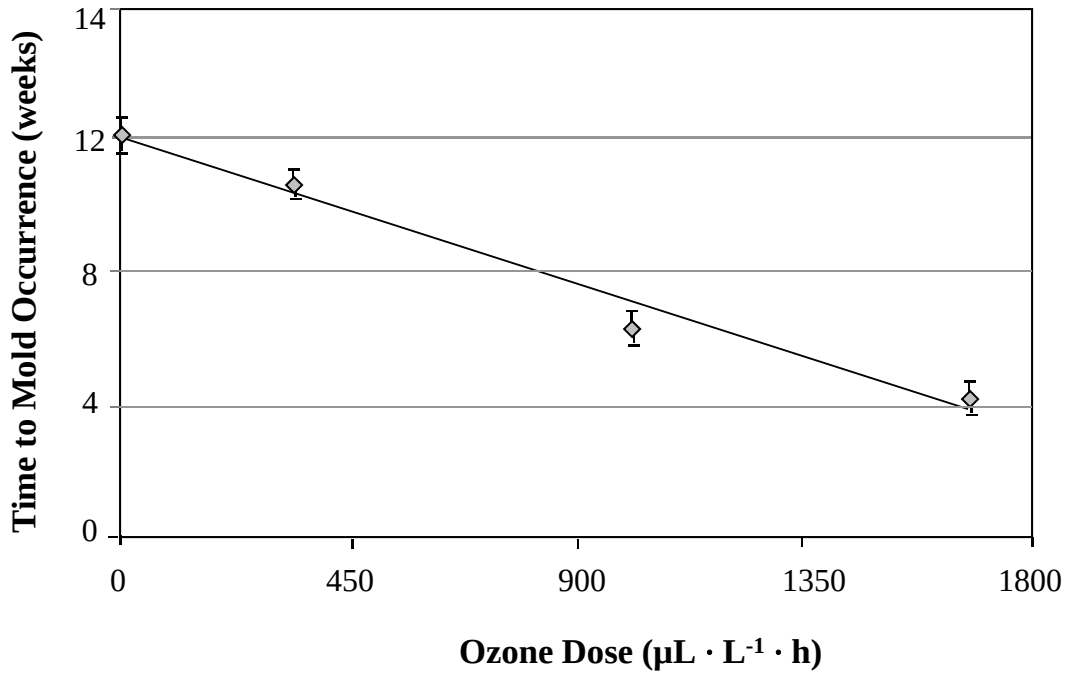

Figure 5. Effects of ozone dose on time to mold occurrence for ozone-treated bamboo incubated at 85 % RH and 30 °C condition. The error bars represent one standard deviation.

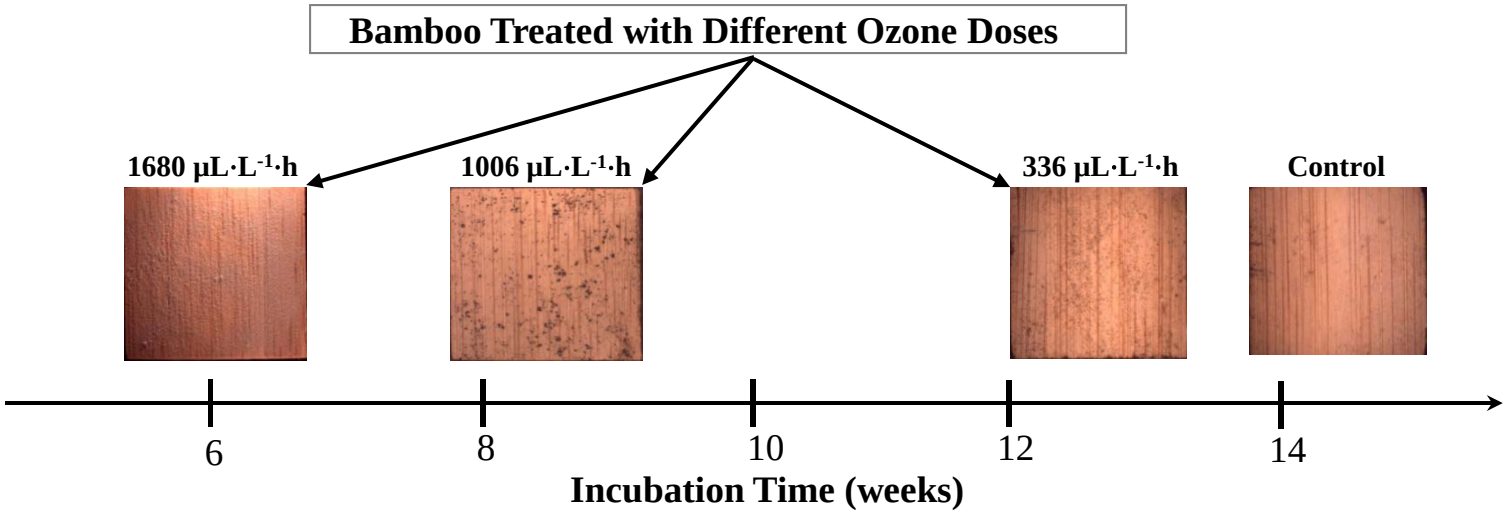

Figure 6. Representative optical microscopy images of entire-surface mold growth versus incubation time for untreated bamboo (control) and bamboo treated with ozone at different doses.