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Mycotoxin production on water damaged building materials

By

Frederick Skrobot III

A Dissertation
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy
in Forest Resources
in the Department of Sustainable Bioproducts

Mississippi State, Mississippi

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Mycotoxin production on water damaged building materials

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Due to the increased occurrence of flooding, biological by-products such as mycotoxins can cause serious health problems for homeowners. This study used two species of fungi, *Aspergillus fumigatus* and *Stachybotrys chartarum*, which have been found within homes after flooding events. Two distinct types of mycotoxins can be produced; gliotoxin by *A. fumigatus* and trichothecenes by *S. chartarum*. A preliminary study evaluated four wall materials separately, for mycotoxin production and validation of techniques. Based on these results, the experimental study built replicated interior walls of gypsum wallboard, fiberglass batt insulation, wood stud, and oriented strand board; placed the walls in mold chambers on concrete pavers; flooded and drained the walls; and exposed these walls to *S. chartarum* for 65 days. All four building materials showed some level of mycotoxin present on all collection days. Gypsum wallboard and fiberglass batt insulation had the highest levels of trichothecenes and levels increased over time. The wood stud and oriented strand board also contained mycotoxins, but levels did not change over time. DNA concentrations were significantly higher on the batt insulation compared to wood products. However, DNA concentrations did not directly correlate to mycotoxin levels. These results support the removal of the batt insulation and

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gypsum wallboard from a home if a flooding event should occur. The finding of the mycotoxin on the wood products requires further research to better understand the mycotoxin's properties on wood in order to ensure the safety of homeowners.

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TABLE OF CONTENTS

ACKNOWLEDGEMENTS ii

LIST OF TABLES v

LIST OF FIGURES vii

CHAPTER

I. LITERATURE REVIEW 1

Introduction 1
Indoor Air Quality and Environmental Factors 2
Fungi and Mycotoxins 5
Mycotoxin Production on Water-Damaged Building Materials 9
Aspergillus fumigatus 11
Stachybotrys chartarum 12
Detection of Mycotoxins 15
Prior Research on Mold in Flooded Walls 18
Objectives of Research 20

II. MATERIALS AND METHODS 21

Preliminary Study: Petri Dish Study 21
Culturing and Inoculation 21
Preparation of Building Materials Samples 22
DNA Extraction 22
Polymerase Chain Reaction (PCR) Amplification 23
Real-Time PCR Procedures 24
Real-Time qPCR 24
RNA Extraction 26
cDNA Synthesis 27
Gene Expression 27
Enzyme-Linked Immunosorbent Assay (ELISA) 29
Thin Layer Chromotography 30
Scanning Electron Microscopy (SEM) 31
Experimental study: Building Wall Study 31
Construction and Inoculation 31
Sample Preparation 36

www.manaraa.com

Statistical Analysis 36

III. RESULTS AND DISCUSSIONS 38

Preliminary Study: Petri Dish Analysis Results 38
DNA Quantification 38
Aspergillus fumigatus 39
Stachybotrys chartarum 42
RNA 46
Mycotoxin Detection 47
Enzyme-Linked Immunosorbent Assay (ELISA) 47
Thin Layer Chromatography 49
Gene Expression 49
Experimental Study: Building Wall Analysis Results 51

IV. CONCLUSIONS 61

REFERENCES 64

APPENDIX

A. METHODS 72

B. RESULTS 77

www.manaraa.com

LIST OF TABLES

3.1 Statistical analysis (P=0.05) of *A. fumigatus* DNA concentrations on the building materials when averaged for each time. 40

3.2 ANOVA (P=0.05) statistical analysis of DNA concentrations of *A. fumigatus* averaged for each collection day on the different building materials.....41

3.3 Statistical analysis (P=0.05) of DNA concentrations of *S. chartarum* as averaged for all building materials. 44

3.4 Statistical analysis (P=0.05) of *S. chartarum* concentrations averaged for each collection day. 44

3.5 Thin Layer Chromatography results of gliotoxin present on different building wall materials.....49

3.6 Analysis of the significant difference (P=0.05) of *S. chartarum* concentrations averaged on different building materials. 54

3.7 Statistical analysis (ANOVA) (P=0.05) averaged for each collection day of *S. chartarum* on the different building materials. 54

3.8 ANOVA (P=0.05) analysis of the significant difference of building wall materials averaged over each collection day.....56

3.9 ANOVA (P=0.05) analysis of the significant difference of each collection day averaged for all materials. 57

3.10 Statistical analysis (ANOVA) (P=0.05) of the average detection of trichothecene concentration on different building wall materials and collection day. 58

A.1 Building materials inoculated with *A. fumigatus* and *S. chartarum* in preliminary study. 73

A.2 Building materials inoculated with *S. chartarum*. 74

B.1 Real-time PCR of *A.fumigatus* DNA concentrations in nanograms per milligram of each building material on selected days. 78

www.manaraa.com

B.2 Real-time PCR of *S. chartarum* DNA concentrations in nanograms per milligram of each building material on selected days. 80

B.3 Experion results for RNA quality for each building material inoculated with *A. fumigatus*. 82

B.4 Experion results for RNA quality for each building material of *S. chartarum*.....84

B.5 Results of ELISA for trichothecenes amounts extracted from different building materials.....86

B.6 Real-time PCR of *S. chartarum* DNA concentrations results in nanograms per milligram of each building material on selected days. 89

B.7 Experion results of RNA quality of building materials. 94

B.8 Results of ELISA for trichothecenes on different building materials.....99

B.9 Trichothecene detection on concrete using an ELISA.....105

www.manaraa.com

LIST OF FIGURES

1.1 The biosynthesis of macrocyclic trichothecenes....14

2.1 Quantitative PCR standard curve of *Stachybotrys chartarum*....25

2.2 Quantitative PCR standard curve of *Aspergillus fumigatus*. 26

2.3 Picture of the interior side of the residential building wall showing the gypsum wallboard.....32

2.4 The back side of the exterior building wall showing the OSB. 32

2.5 Picture of the inside of the residential building wall showing the wood studs and batt insulation.....33

2.6 Paver stone at the bottom of the mold chamber....33

2.7 Residential wall placed into container 34

2.8 The mold chamber with the filter for air exchange and the wall sitting on top of the paver stone.....35

3.1 *Aspergillus fumigatus* DNA concentrations in nanograms of DNA per milligram of material on different building wall materials.....39

3.2 Micrograph of *Aspergillus fumigatus* on Day 65 on plywood building material sample. 41

3.3 Micrograph of *Aspergillus fumigatus* on Day 65 on oriented strand board material samples. 42

3.4 The distribution of *Stachybotrys chartarum* DNA concentration in nanograms of DNA per milligram of material on different building wall materials.....43

3.5 Scanning electron microscopy micrograph of *S. chartarum* on oriented strand board on day 65.....45

3.6 Scanning electron microscopy micrograph of *S. chartarum* on oriented strand board on day 65.....45

www.manaraa.com

3.7 ELISA results for trichothecene detection with standard error bars. 48

3.8 Gene expression on selected building material and day amended with the 18S housekeeping gene. 50

3.9 Picture of dismantled wall on day 65. 51

3.10 Picture of gypsum wallboard surface layer on extraction day 65 52

3.11 Picture of gypsum wallboard bottom brown paper (side facing the insulation) side on extraction day 65 52

3.12 *Stachybotrys chartarum* concentrations on building wall materials in nanograms of DNA per milligram of material on different building materials. 53

3.13 Mycotoxin concentration in ppb on different building materials as measured by ELISA. 55

3.14 ANOVA (P=0.05) analysis of the significant difference of each building material averaged for all collection dates. 56

3.15 Statistical (ANOVA) (P=0.05) analysis of the significant difference of trichothecene concentration on each day averaged for all materials. 57

3.16 Trichothecenes on the concrete slab on different days as determined by ELISA. 59

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CHAPTER I

LITERATURE REVIEW

Introduction

The effect of climate change can influence hurricane intensification, sea level rises, and storm surge, which influences the extent of flooding, the number of people affected, and amount of property damage (Frey et al. 2010). Water damage caused by flooding can enter a home through many passages and can be absorbed by the building materials. The conditions that impact the growth and development of a microbial organism include the design of the building wall, the drying ability of the building wall materials, and presence of nutrients after flood waters have receded (Taylor et al. 2011).

Indoor dampness and mold problems are common around the world, and are one of the most important indoor problems worldwide. In fact, it has been shown from multiple sources that the percent of buildings with mold-contamination in the United States and Canada is about 36%, while in the Scandinavia countries the mold and dampness in buildings were reported as approximately 15 and 24% respectively (Verhoeff et al. 1990; Spengler et al. 1993; Pirhonen et al. 1996). In cold climates, the occurrence of water damage and dampness problems has been very low while in moderate and warm climates the estimates have been very high (Quansah et al. 2012). The indoor air quality can have an impact on our health and well-being. In 2004, Bornehag et al. stated "that people living and working in moldy buildings are more prone

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to respiratory symptoms and diseases than people in non-problem buildings and thus provided evidence for a true association between dampness and health" (Polizzi et al. 2009). Interestingly, fungal spore concentration in indoor air can be influenced by the age of the building. Buildings are now constructed with different types of materials and all of these materials can potentially provide a nutrient source and environmental conditions for different microorganisms, in particular fungi, to grow. However, the different materials will likely support different fungal species as well as different rates of growth Sivasubramani et al. (2004).

Molds can cause adverse human health effects through specific mechanisms such as causing a harmful immunological response or toxic-irritant effects from mold byproducts directly (Bush et al. 2006). The emissions produced by some molds are a potential health risk for respiratory infections and severity of symptoms. The illnesses that cause severe symptoms due to dampness and molds have been reported among people who range in age from small children to adults in buildings located worldwide (Pirhonen et al. 1996).

Indoor Air Quality and Environmental Factors

Floods, such as the 2011 Mississippi River floods, which were the most damaging river floods recorded in the last century, as well as the devastation from Hurricanes Katrina and Rita in 2005 and superstorm Sandy in 2012, can introduce up to 2 m of standing water into a home. In the wake of Hurricanes Katrina and Rita, numerous studies evaluated flood-damaged homes for elevated levels of mold spores, culturable fungi, and byproducts from the organism (Chew et al. 2006; Solomon et al. 2006; Rao et al. 2007; Riggs et al. 2008; Adhikari et al. 2009; Bloom et al. 2009). Riggs et al. (2008)

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evaluated 112 homes in New Orleans after the hurricanes and found that 64% were flooded, with 19% flooded with greater than 1.8m of water, 19% flooded with 0.9-1.8m of water, and 26% flooded with less than 0.9m of water. The scientists found that visible mold, culturable mold, and identified species spore numbers were all significantly greater in homes with flood levels higher than 0.9m. Shelton et al. (2002) stated "that fungi usually enter a building through outdoor air sources such as heating, air conditioning systems, and through doors and windows." They also found that the most culturable airborne fungi, both indoors and outdoors and in all seasons and regions, were *Cladosporium, Penicillium*, nonsporulating fungi, and *Aspergillus*. *Stachybotrys chartarum* was identified in the indoor air in 6% of the buildings studied and in the outdoor air of 1% of the buildings studied.

It is estimated that people spend approximately more than 90% of their time indoors; indoor air quality control is needed to protect occupants from potential health risks (Choi and Edwards 2012). There is strong evidence that moisture related problems in buildings increase the risk for health effects among adults and children both in their homes, public buildings, and office buildings. The most important health effects that are associated with dampness in buildings seem to be symptoms involving the respiratory system (Schleibinger et al. 2008). A recent report from the Institute of Medicine of the National Academies (2004) found sufficient evidence to conclude that there is an association between indoor-dampness-related agents and asthma, upper respiratory tract symptoms, coughing, and wheezing. They estimate that about 20% of current asthma in the United States can be attributed to dampness in the home. In addition, symptoms may

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occur more frequently in individuals exposed to moldy conditions (Institute of Medicine of the National Academies 2004).

During the energy crisis in the 1970's, construction procedures changed and buildings were tightly sealed, ventilation rates reduced, and new materials introduced to minimize energy loss. In fact, within this same decade, illnesses associated with poor indoor air quality were reported in the medical and scientific literature (Sundell 2004). One of the main environmental factors that influences spore and particle release from fungal structures growing on surfaces is relative humidity (Frankel et al. 2012). Air contains a certain amount of water vapor, but moisture amounts in vapor content depend on temperature. Relative humidity is defined "as the current water vapor content in relation to the vapor content at saturation, represented as a percentage" (Johansson et al. 2012). In relation with ambient air, materials can absorb moisture, or they can release moisture, and reach an equilibrium moisture content (EMC) with their environment. Water activity is defined as "the ratio between the vapor pressure of the building material itself and the vapor pressure of distilled water under the same conditions and it is expressed as a fraction" (Flatt 2002) Wood is a hygroscopic material, which allows wood products to exchange water vapor with the surrounding air until they reach EMC. However certain products such as oriented strand board (OSB) have resins and adhesives which restrict water movement, thus the product equalizes at lower moisture content than dimensional lumber. The amount of moisture in a product will allow some molds to produce spores and even produce mycotoxins. Nielsen (2003) states that "fungal growth in buildings starts at a water activity near 0.80 but significant quantities of mycotoxins are not produced unless water activity reaches 0.95." The accumulation of dirt, soil, and

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dust on building materials can actually serve as nutrients and enable microorganisms to grow at a lower water activity (Pasanen et al. 2000). Different building materials are prone to different molds. For example, the water activity of the penicillia and aspergilli depends on the composition of the building material. Wood, wood composites such as plywood, oriented strand board, and other materials with high cellulose content are capable of supporting mold growth at the lowest water activity values; while plasterboard reinforced with cardboard and paper, or inorganic materials coated with paint or additives that offer an easily-degradable carbon source, will not support growth unless water activity is between 0.85-0.9. (Nielsen 2003). Molds growing on building materials can be divided into three groups based on their water activity requirements on laboratory substrates (Grant et al. 1989), and responses to changes in water activity (Nielsen 2003). There are primary colonizers, which are capable of growing at water activity of less than 0.8, secondary colonizers, requiring water activity between 0.8 and 0.9, and tertiary colonizers or water-damage molds, which need a water activity greater than 0.9. These latter molds are also known to be the most toxic species of fungi. Interestingly materials made of stone such as concrete and brick support fewer mold although surface dust could provide additional nutrients (Viitanen et al. 2010). Andersen et al. (2011) found that *A. fumigatus* and *Chaetomium* spp on concrete and flooring materials were able to sporulate and tolerate alkaline conditions upon flooding.

Fungi and Mycotoxins

In the 18th century, Linnaeus classified fungi as organisms belonging to a subgroup within the plant kingdom. Until a few decades ago, fungi were grouped with "cryptogams"(plants without flowers or seeds). True fungi have been split off from other

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heterotrophic cryptograms such as the slime molds and Oomycota and are placed in a kingdom of their own (Barr 1992; Baldauf and Palmer 1993; Alexopoulos et al. 1996). Fungi are made up of vegetative mycelia that consist of a large number of branched hyphae and are able to become airborne and inhaled very easily. Spores are often released by different mechanisms, which extend the livelihood of the species (Després et al. 2012). In order for the fungi to survive, factors such as food sources, temperature, and moisture are very important. (Borkovich and Ebbole 2010). Moisture accumulation from different sources such as leakages, condensation or inadequate insulation often promotes microbial growth in built structures leading to harmful emissions that can impact indoor air quality (Koskinen et al. 1999). It has been mentioned from multiple sources that mycotoxins were "found to be among the oldest environmental toxicants to cause humans problems since ancient times" (Kampelmacher 1973; Niessen 2007). The name mycotoxin is a combination of the Greek word for fungus 'mykes' and the Latin word 'toxicum' meaning poison (Turner et al. 2009). Mycotoxins are defined "as natural, chemically diverse, secondary metabolites produced by different filamentous fungi that can have various health effects on humans ranging from acutely toxic to immunosuppressive or carcinogenic." These secondary metabolites are produced on the outside of the organism and aids in the protection of the microorganism from its environment (Fisvad et al. 1998). Unfortunately homeowners that come in contact with mycotoxins through inhalation, ingestion, or skin contact can develop serious health risks. The production of a particular mycotoxin can be limited to a certain number of fungal species, and in some instances, are limited to particular strains. These products are usually produced when nutrients for growth are scarce (Hintikka and Nikulin 1998;

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Mostafa et al. 2012). Trichothecene mycotoxins for example, produced by *Stachybotrys chartarum*, have been associated with health problems for people living or working in contaminated buildings (Nieminen et al. 2002). Therefore it is now known that there is a relationship between indoor molds, building related illnesses due to the exposure to spores, and the production of mycotoxins (Betancourt et al. 2013).

The US Environmental Protection Agency has developed the Environmental Relative Moldiness Index, which classifies moldiness based on the presence of 36 mold species. These species are divided into two groups. Group 1 contains 26 species of molds that are associated with both water intrusion and significant adverse health impacts, while Group 2 contains 10 species of common indoor molds that do not appear to be associated with either negative health effects nor associated with water intrusion. It is also worth noting that the molds listed in Group 1 produce mycotoxins that can be transported to humans in or on the fungal spores and fragments (Vesper et al. 2007). In addition to mycotoxins, by-products such as volatile organic compounds (VOC) are also produced by molds,as well as β-(1-3)-D- glucans. All of these released products can severely impact human health within water-damaged homes, resulting in flu-like illnesses and fatigue (Rylander et al. 1998). Fungi, rather than bacteria or viruses, are the principal biological contaminants within the home that are responsible for the health problems (Menetrez et al. 2009). The most significant genera, posing a major health hazard to humans include *Alternaria, Aspergillus, Cladosporium, Fusarium, Pencillium, Rhizopus*, and *Stachybotrys*. A study conducted by Lawton et al. (1998) evaluated the moisture and fungal problems in 59 houses located in southern Ontario and found visible fungal growth on building materials as well as in dust and air. Fungal growth was hidden in

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building wall envelopes, which they suspected was the largest source of fungal growth, followed by ceilings of bathrooms, and refrigerator drains. It has been estimated in 1999, mold contamination affected 25% of Polish flats, and about eight million people in were exposed to mycotoxins and allergens produced by molds growing on building materials (Gutarowska and Piotrowska 2007).

Air-handling systems may contain different microorganisms such as molds that are capable of producing mycotoxins. These airborne molds could be inhaled and cause different types of symptoms such as cough, irritation of eyes, and dermal irritation (Tuomi et al. 2000; Mentrez et al. 2009). Molds can produce over 300 mycotoxins and environmental factors may stimulate toxin production (Gutarowska and Piotrowska 2007). Even though some fungi can grow on almost any natural or synthetic construction material, mycotoxin production depends on environmental conditions, pH, and the type of material (Hintikka and Nikulin 1998).The structure of mycotoxins range from a simple C_4- compound to complex structures. An example of a complex mycotoxin are the trichothecenes, which contain a rigid tetracyclic ring with a cyclohexane, A-ring with a double C-C bond between C-9 and C-10, a tetrahydropyranyl B-ring, a cyclopentyl C-ring, and an epoxide at C-12 and C-13 (Shank et al. 2011). The epoxide at C-12 and C-13 allows for the macrocyclic structure to become aerosolized.

It has been suggested that some of the fungal strains that produce toxic secondary metabolites, become airborne by microbial spores and cell fragments, and thus impact occupants through inhalation of indoor air once passed the alveoli of the lung. These fragments from water-damaged building materials may increase the risk of mycotoxin exposure and cause irritation symptoms such as cough, rhinitis, hoarseness, and

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wheezing. Recurrent respiratory infections such as allergic alveolitis, sarcoidosis and rheumatic disease have been linked with moisture damage in indoor environments (Taubel et al. 2011). It has been estimated that "there are 21.8 million people with asthma in the United States and that approximately 4.6 million cases can be attributed to dampness and mold exposure in homes" (Mudarri and Fisk 2007).

Mycotoxin Production on Water-Damaged Building Materials

Wood products placed into buildings are perfect environments for mold growth depending on humidity and nutrient availability (Nielsen 2003). Some molds secrete secondary metabolites, such as mycotoxins, during the stationary phase of growth (Moss 1996). As stated previously, the production of a mycotoxin is generally confined to a relatively small number of fungal species and could be species or strain specific. Mello and Macdonald (2003) state that there is convincing evidence to suggest that "the more intricate the pathway of synthesis of a particular mycotoxin, the fewer the number of fungal species produce that compound." Numerous reports have been published on the effects of mycotoxigenic fungi on the indoor environment, but none of them address the mycotoxins production and other metabolites that molds generate in buildings. According to Kuhn and Ghannoum (2003) mycotoxins are only developed when stressful environmental conditions are occurring or nutrients are near depletion. Also the presence of toxigenic fungi doesn't imply the presence of mycotoxins, nor does the finding of mycotoxins prove that a particular species was present. The ability for a mold species to produce mycotoxins is determined by many factors. However, very few studies have established a relationship between mycotoxin exposure and building-related illness. In

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fact, air and material samples taken indoors rarely have detected mycotoxins (Tuomi et al. 2000).

The transport of spores and other particles from the surfaces of moldy building materials is an important determinant of the exposure of people working and living in buildings that have water damage. The release of spores depends on a number of factors including the type of material, the fungal species, relative humidity, temperature, age of mycelia, and disturbances of the building materials (Kideso et al. 2003). Fungi can release spores into the air by an active mechanism or passive mechanism. The active mechanism requires the fungus to provide the energy needed for spore release, while the passive mechanism relies on air currents, rain, or gravity. It is interesting that passive spore release is most common for fungi in indoor environments (Gorny et al. 2001).

Damp or water-damaged building materials are at high risk of fungal growth, which may result in health problems for the occupants. In an environmental evaluation beginning in November 1998, scientists conducted experiments including material and air sampling in a 10-story hotel. Rooms within the hotel, which had severe water damage but not remediated, were chosen for sampling. The predominant fungi including *Acremonium sp*., *Alternaria sp*., *Aspergillus niger*, *Aspergillus sp*., *Mucor sp*., *Penicillium sp*., *Phoma sp*., *Stachybotrys chartarum*, and yeasts were collected on material samples. Among the identified fungi, *Aspergillus sp., Penicillium sp*., and *Stachybotrys chartarum* are capable of producing mycotoxins (Trout et al. 2001). *Aspergillus fumigatus* and *Stachybotrys chartarum* have been associated with building construction materials (Andersen et al. 2011). These authors found that *A. fumigatus* was isolated from the concrete, which may be introduced by dirt and is able to survive harsh environmental conditions. The other

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organism, *S. chartarum* was found on gypsum wallboard and can be difficult to detect during both air and dust sampling.

Aspergillus fumigatus

Pier Antonio Micheli first described the genus in 1729 in *Nova Plantarum Genera* (New Genera of Plants) and named it *Aspergillus* for its resemblance to a holy water sprinkler or aspergillum (Mackenzie 1988). *A. fumigatus* is considered to be saprophytic and opportunistic pathogenic filamentous fungus, which is naturally found in the soil where it survives and grows on organic debris. *A. fumigatus* produces green echinulate conidia (2.5 to 3µm in diameter), in chains. This fungus is thermophilic, with growth occurring at temperatures as high as 55ºC and surviving temperatures up to 70ºC. It is an abundant sporulater producing over 50,000 conidia per conidiophore. *A. fumigatus* relies on disturbances of the environment and strong air currents to release its spores. Once the conidia are in the air, their small size allows for them to be buoyant, keeping them airborne both indoors and outdoors. The growth characteristics of this organism such as high spore concentration in the air and its faster growth relative to other airborne fungi at 40ºC are major contributors to its virulence (Bok et al. 2006).

The main portal of entry for this organism into a host species is the respiratory tract system, which due to repeated exposure to conidia and antigens could cause allergic responses including asthma, allergic sinusitis, and alveolitis. However this organism can also cause allergic bronchopulmonary aspergillosis, aspergilloma, and life-threatening systemic disease called invasive aspergillosis. These severe syndromes are most likely found in immunocompromised individuals and are a complication in leukemia sufferers, organ transplant recipients, and HIV-AIDS patients (Panaccione and Coyle 2005). In

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particular invasive aspergillosis has increased due to an increased population of immunosuppressed patients (Latge 1999).

A. fumigatus is frequently isolated from moldy buildings and especially located in dust particles (Nielsen 2003). This organism can produce many different mycotoxins, however the main mycotoxin that impacts water-damaged homes is gliotoxin. A study conducted by Nieminen et al. (2002), which isolated and purified both genotoxic and cytotoxic compounds from the culture medium of *A. fumigatus*, found that gliotoxin wasn't associated with spores or mycelia but was rather absorbed into the building materials. Gliotoxin is a highly toxic mycotoxin that can be produced by several filamentous fungi and impact water damaged homes. It is an "epipolythiodioxopiperazine (ETP), a class of cyclic dipeptides characterized by the presence of an internal disulphide bridge" (Gardiner and Howlett 2005). Very little is known about the biosynthesis of ETP, however it is now known that all natural ETPs that were successfully isolated contain at least one aromatic amino acid and the diketopiperazine ring, which is derived from a cyclic dipeptide. The genes responsible for biosynthesis of gliotoxin in *A. fumigatus* were identified as a cluster of 12 genes (Gardiner and Howlett 2005). The reported modes of "toxicity include: 1) act as redox active toxins generating reactive oxygen species by cycling between their oxidized (disulfide) and reduced (dithiol) forms and 2) they are able to form mixed disulfides with proteins that have accessible thiol groups" (Munday 1987; Chai and Waring 2000).

Stachybotrys chartarum

Stachybotrys chartarum is ubiquitous fungus that is able to colonize within water damaged buildings. This fungus is often found on water-saturated cellulose such as

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gypsum board and wallpaper and can be considered an indicator for water intrusion (Yike et al. 2006). This black mold produces a mass of sticky, single celled ornamented conidia from phialides on each conidiophore. The growth of this organism requires a high moisture content, water activity greater than 0.9, thus grows well where moisture has accumulated. However, this organism is found in low concentrations among fungi identified in water-damaged buildings. This organism can produce two mycotoxin chemotypes (Koster et al. 2003). One chemotype leads to the manufacture of the toxic macrocyclic trichothecenes, whereas the second chemotype produces non-toxic the simple trichothecenes (Petska et al. 2008). The members of the macrocyclic trichothecenes family of mycotoxins are known to be potent inhibitors of protein synthesis in eukaryotes.

The first isolation of trichothecenes was by Eppley and Bailey in 1973 from *Stachybotrys chartarum*, in which they were able to classify four different categories of trichothecenes. Figure 1.1 shows the purposed biosynthesis of macrocyclic trichothences from Nielsen (2002). The most important step in the biosynthesis of trichothecenes as stated by Nielsen (2002) is the "cyclization to trichodiene by trichodiene synthase, which is encoded by the TRI5 gene." After the cyclization, the macrocyclic compound, Roridan E acts as a precursor for the other macrocyclic compounds. These other macrocyclic mycotoxins include Verrucarin J, Verrucarin B, Satratoxin G&F, and Satratoxin H. All of these compounds can occur in the outer plasmalemma surface and the inner wall layers of conidiospores. In fact, satratoxin G was found to be localized primarily in the conidia, followed by the phialides and hyphae. Also these compounds can be aerosolized and cause major health risks for individuals if inhaled.The ability for *S. chartarum* to produce

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biologically potent mycotoxins and its previous association of mycotoxicoses in animal studies has resulted in this fungus being referred to as “toxic black mold” (Pestka et al. 2008).

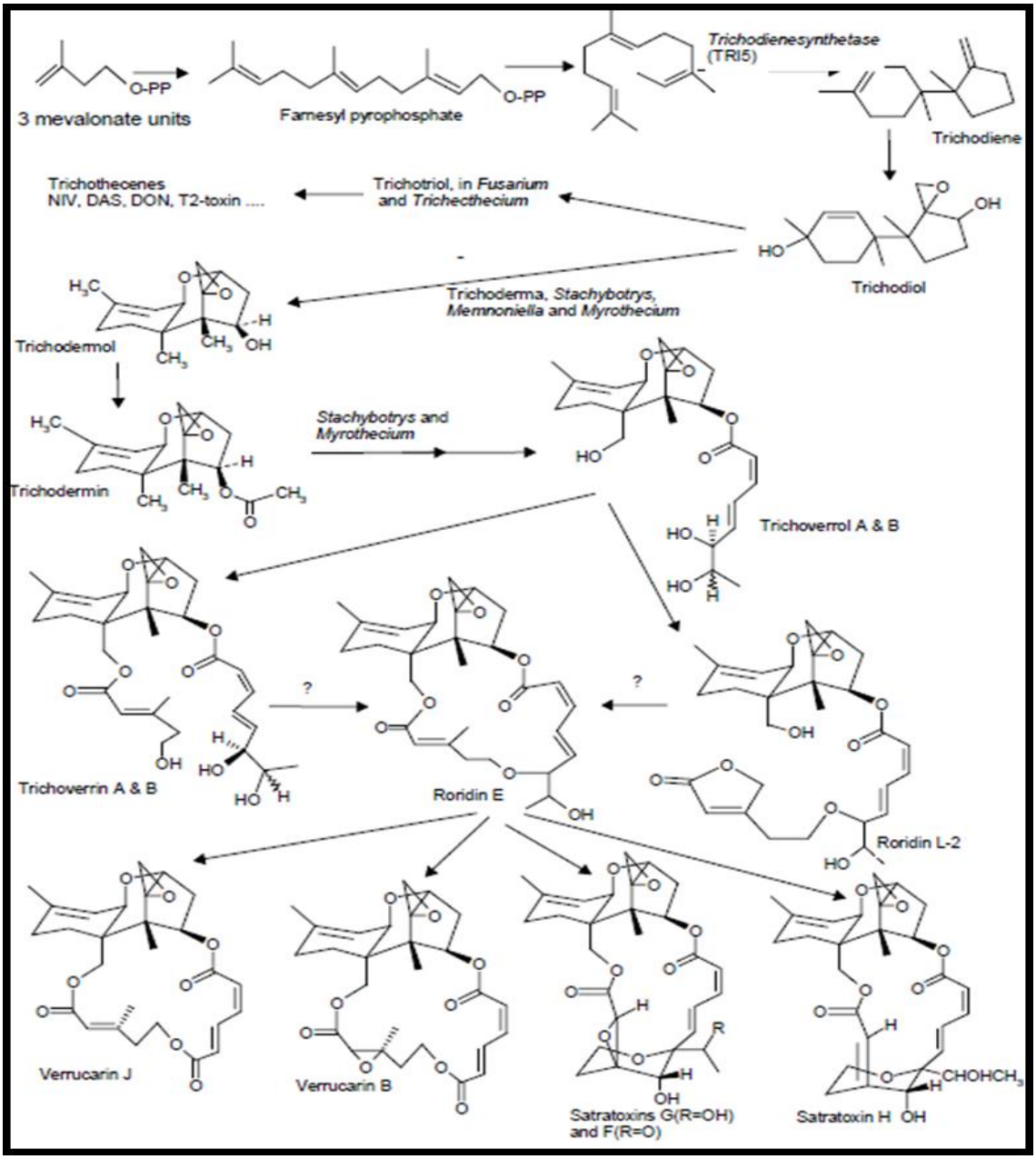

Figure 1.1 The biosynthesis of macrocyclic trichothecenes from Nielsen (2002).

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It has been shown that trichothecene mycotoxins produced by *S. chartarum* have been associated with serious health effects in people living or working in buildings infested with this fungus. It is very difficult to detect the mycotoxin since the toxin is produced in a slimy mass with high moisture content, becoming airborne only when dry and aerosolized when attached to other particles such as dust. Therefore the use of an air filter or other air collection methods to detect this mycotoxin is likely inaccurate (Nieminen et al. 2002; Kuhn and Ghannoum 2003). These mycotoxins can bind to a single site on eukaryotic ribosomes and directly inhibit protein synthesis, depending on the toxin. The presence of *S. chatarum* mycotoxins was reported to have caused pulmonary hemorrhage in exposed infants (Menetrez et al 2009). The mycotoxin stachylysin is more likely to be found in strains of *S. chartarum* isolated from homes of children with pulmonary hemorrhage and hemosiderosis (Vesper et al. 2000). Jarvis et al. (1998) reviewed studies on the different chemotypes of *S. chartarum* and concluded that about one-third of *S. chartarum* isolates produce the toxic macrocyclic trichothecenes and about two-thirds produce the simple nontoxic trichothecenes.

Detection of Mycotoxins

Fungal growth in damp or water-damaged buildings worldwide is an increasing problem, which has adverse effects on both the occupants and the buildings. A fungus is able to proliferate when in favorable conditions and may secrete mycotoxins. The severity of the toxin varies and depends on the individuals affected, based on susceptibility, metabolism, and defense mechanisms. The techniques for detecting known mycotoxins are quite advanced and range from directly sampling for the toxins, methods

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based upon physical characteristics of the toxins, and methods such as immunoassays (Maragos and Busman 2010).

The detection of mycotoxins can be determined through either analytical or molecular techniques. Some of the earliest analytical methods for detecting mycotoxins were chromatographic and chromatographic assay, which have continued to improve with advancements in instrumentation. For the detection of mycotoxins in the analytical laboratory, the most common methods used today are chemiluminscent immunoassay and bioluminescent assays. These methods are considered to be alternatives to the conventional methods. The analytical testing of mycotoxins based on chromatography or immunoassays usually require a solvent extraction to the mycotoxin, and the subsequent clean-up of the extract to reduce "matrix effects". This is usually accomplished using various combinations of solvents, with the addition of modifiers (acids, bases, etc.) for extraction, and depends on the properties of the mycotoxins and the sample (Krska et al. 2008). Conventional analytical methods include high performance liquid chromatography (HPLC), thin layer chromatography (TLC), gas chromatography, and mass spectrometry (GC-MS). HPLC uses various adsorbents and separation depends on the physical and chemical structure of the mycotoxin. HPLC can detect multiple mycotoxins within a single chromatographic run, however, the components of a sample matrix can interfere with the ability to quantify the sample accurately (Margos and Busman 2010). Gas chromatography and mass spectrometry are methods used to confirm the identity of different mycotoxins. Nielsen et al (1998) used gas chromatography for the analysis of trichothecene from water- damaged gypsum boards in Danish buildings and trichodermol and verrucarol. This method proved to be very advantageous compared to other methods.

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However the main problems using gas chromatography are "increased trichothecene response, non-linearity of calibration curves, drifting responses, carry-over or memory effects from previous samples and high variation in reproducibility and repeatability" (Pascale 2009).

The detection of mycotoxins in the molecular laboratory involves using genomic DNA sequences, which will allow for the identification of species responsible for producing specific mycotoxins. PCR can also be used with reverse transcriptase to detect genes that are being expressed rather than simply detect the presence of the DNA that encodes a particular gene. Reverse transcriptase generates a DNA copy from RNA (mRNA) that is present when the gene is being expressed. The complementary DNA (cDNA) amplified with primers targeting the mycotoxin gene sequence (Mostafa et al. 2012)The cDNA identify genes and gene clusters that are expressed during biosynthesis of specific mycotoxins. Sequencing of the amplified fragments confirms the identity of the toxin biosynthetic genes. By using real-time reverse transcriptase PCR (RT-PCR), "Cramer et al. (2006) identified the gene, called gliP, for production of gliotoxin in the genome of *A. fumigatus*." A disruption of the gene resulted in the termination of gliotoxin production.

The most common and commercial means for detection of mycotoxins are based on the competition enzyme linked immunosorbent assay such as ELISA which have been available since the 1970s" (Pascale 2009; Stark 2010; Rai et al. 2012). The assays can be conducted in formats with either the test antigen or the antibody immobilized. ELISA tests are rapid, usually do not require extra clean-up steps, however, lack accuracy at low concentrations. (Krska et al. 2008; Pascale 2009).

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In 1982, The World Health Organization made the first attempt to define "sick building syndrome" (Danna and Griffin 1999). However, the set of conditions leading to this have proven to be difficult to define, and no single cause has ever been identified. It has been suggested that fungi and their secondary metabolites, such as mycotoxins, are contributors to sick building syndrome. In addition to airborne conidia, mycelial fragments and dust can also be aerosolized and carry mycotoxins with them (Brasel et al. 2005). The analytical methods for the determination of mycotoxins have improved. However, detection of mycotoxins can still be very difficult.

Prior Research on Mold in Flooded Walls

Prior research focused on the types and quantities of mold species that could exist within the walls of a flooded home (Skrobot et al. 2014). A small 'flood unit' was built to residential code, and flooded for three weeks using farm land pond water. The pond water was then drained and the unit remained closed for another three weeks. The wall materials included wet samples and dry samples approximately seven months after opening and removed from the flood unit. Wall materials, including both above and below the water line materials, were removed from various locations. The wet material samples included four samples of gypsum wallboard, fiberglass batt insulation, wood studs, wood sheathing, vinyl siding, and house wrap. The dry materials were comprised of gypsum wallboard and wood studs.

The authors identified on the wet wall materials exposed above the water level, 14 different fungal species, and 11 species were identified on the wet wall materials exposed below the flood water. A total of eight species were identified from the materials that had been dried for approximately seven months. There were two species in common between

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the dry above the water line and the wet above the water line, *Aspergillus niger* and *Fusarium oxysporum*, and no species in common between the dry and wet below the water line. *A. fumigatus* and *S. chartarum* were identified more often than the other fungi on both above and below the water line on the dry materials.

Real-time PCR was used to quantitate the different types of molds that were identified and selected within the flood unit materials. The Group 1 mold species that were selected included *A. fumigatus*, *A. niger*, *Aspergillus terreus*, *Aspergillus versicolor*, *Chaetomium globosum*, *Paecilomyces variotii*, and *S. chartarum*. *A. fumigatus* was found in the highest concentration of fungi measured on both the wet and dry wall materials followed by *C. globosum* and *Trichoderma viride*. *Trichoderma* were found only in the wet materials and were absent from the dry material samples. *A. fumigatus* was found in the highest concentration of any of the seven fungi measured on both the wet and dry wall materials. This species was found primarily on the batt insulation below the water line and on the dry materials. These dry materials included only the wood stud. *S. chartarum* was found in high concentrations on the wet materials particularly on the batt insulation and on a sample of gypsum wallboard. However this organism was found in low levels inside the flood unit by air sampling after re-entry and after drying. Interestingly in this study, the batt insulation supported the greatest concentration of fungi in particular *A. fumigatus* and *S. chartarum*. This compared with all other wall materials followed by the paper sidings of the gypsum wallboard and then the plywood sheathing. The solid wood studs, vinyl siding, and house wrap supported very low concentrations of mold. Overall, the dry gypsum contained high levels of mold and was dominated by *S. chartarum*. The high concentrations and the severity of this species were

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surprising after seven months after flooding. The vinyl siding and house wrap contained moderate levels of *A. fumigatus*, however it is believed that these housing components contribute little to the mold population after flooding. Based on these findings, *A. fumigatus* and *S. chartarum* were chosen as the fungal species for the research presented here.

Objectives of Research

The objective of this investigation was to measure mycotoxin gene expression levels produced by *Aspergillus fumigatus* and *Stachybotrys chartarum* on batt insulation, wood stud, wood sheathing, and gypsum using reverse transcription PCR (RT-PCR) under simulated flood conditions. The specific aims were to (1) determine which building materials support the highest production of mycotoxins, (2) determine under what environmental conditions the genes for mycotoxin production are being expressed, and (3) determine the concentration of mycotoxins being produced.

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CHAPTER II

MATERIALS AND METHODS

Preliminary Study: Petri Dish Study

Culturing and Inoculation

The fungal species *Aspergillus fumigatus* and *Stachybotrys chartarum* were both cultured on Potato Dextrose Agar (PDA). Plates were plugged and incubated for approximately one week. Once fungal mycelia were visible, approximately 1mL of distilled water was placed on top of the mycelium and, scraped sixty-five plates of each species were lightly scraped to dislodge the spores. The water was removed from each plate and pooled to a total of 60mL. The spore mixture was put into a 125mL flask and the contents agitated using a stir bar for approximately 5 minutes. Fifty ml of this spore mixture the mixture was placed into the glass spray dispenser. A subset of this mixture used to estimate inoculum level using a hemocytometer. These concentrations were $1.19E^{05}$ cells/ml for *A.fumigatus* and $1.28E^{05}$ cells/ml for *S. chartarum*.

The interior building wall component tested for fiberglass batt insulation, cellulose batt insulation, plywood sheathing (12.7mm x 49.19mm x 9.52mm), and oriented strand board (12.7mm x 49.19mm x 9.52mm). These materials were heat treated at 100 ° C for one hour. Each building material was placed into petri dishes and inoculated directly by using a glass spray unit (Sigma, St. Louis, MO, 50mL) with compressed air. Approximately 0.5mL of suspended inoculum of either *A. fumigatus* or *S.*

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chartarum was sprayed onto each sample. Sample codes for each building wall materials are listed in Appendix A: Methods (Table A.1). After inoculation, the petri dishes were placed inside an incubator at a temperature of 28°C. After incubation, each sample was tested for mold growth and gene expression for gliP (gliotoxin) and TRI5 (trichothecenes) at 15, 35, 50, and 65 days. Procedures were modified from Nielsen et al. (1999). There were five replicates processed for each building material at each sampling day. Thus a total of 160 samples were examined in this study. Eighty samples were inoculated with *A. fumgiatus* and eighty inoculated with *S. chartarum*.

Preparation of Building Materials Samples

When removed from incubator on the appropriate day, the batt insulation was already cut to size and placed into a 2mL capped tube with two sterile 5mm beads, while the wood sheathing (OSB and plywood) were reduced to smaller sizes using wood gouges under cold room conditions (2°C) and placed in storage bags. After wood sheathing was reduced to size, each building material sample was weighed and placed into 2mL capped tubes with two sterile 5mm beads in the laboratory. Each building material listed in Table A.1 was stored at -70°C until processing.

DNA Extraction

DNA extraction was conducted using the Nucleospin Plant II Kit (Machery Nagel, Duren, Germany), which included 800µl of CTAB lysis buffer (2% cis-trimethyl ammonium boric acid, 100mM Tris, 20mM Na_2EDTA, 1.4 M NaCl, and 1% polyvinylpyrolidine, pH 8.0) and 20µl RNase A. Samples were placed onto a Biospec Mini Beadbeater bead mill (Bartlesville, OK) for three minutes. After two cycles for

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three minutes at maximum speed on the bead mill, the samples were placed in a water bath at 65º C for two hours. DNA was extracted following Machery Nagel kit protocol. DNA samples were eluted with 80µl of preheated 70 º C PE buffer. All samples in the second building wall study were freeze dried for one hour.

Fungal genomic DNA from either *A. fumigatus* or *S. chartarum* was extracted following the Nucleospin Plant II Kit (Machery Nagel, Duren, Germany) protocol for fungi. Both species of fungi were isolated from a previous study (Skrobot et al. 2014). DNA purity was assessed by gel electrophoresis and concentrations determined with a Nanodrop 1000 spectrophotometer (Thermo Scientific, Waltham, MA) and found to be 17.8 ng/ml for *A. fumigatus* and 23.2 ng/ml for *S. chartarum*.

Polymerase Chain Reaction (PCR) Amplification

After DNA extraction, each sample was amplified using an Eppendorff Thermocycler (Hamburg, Germany) to analyze the quality of DNA present for each material sample. The eluted DNA (2µL) in addition to 8µL Ultra pure water (Sigma, St. Louis, MO) was added to a 0.2µl PCR tube and placed in a thermocycler for a four minute 98°C hot start. Once the four minutes were met, 40µl of Master Mix consisting of 25mM MgCl (Fisher, Grand Island, NY), 10mM Buffer (Fisher, Grand Island, NY), 5 units/µl Taq DNA polymerase enzyme, and 10mM ITS1-forward (5`GTAGTCATATGCTTGTCTC-3`), and 10mM ITS4-reverse (5`CTTCCGTCAATTCCTTTAAG-3`) primer, described by White et al. (1990), was added with the Taq polymerase into each PCR tube and placed in a thermocycler using 45 seconds at 95ºC for denaturing, 45 seconds at 52ºC for annealing, and 2 minute at 72ºC for extension for 35 cycles with a final 10 minute 72ºC extension to amplify the

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DNA. Once the DNA was amplified, the product was analyzed by gel electrophoresis. Gel electrophoresis was run on a 2% agarose gel in Sodium Boric Acid (SBA) buffer, with GelStar or GelRed dye.

Real-Time PCR Procedures

Real-Time qPCR

Quantitative Polymerase Chain Reaction (qPCR) was used to determine the quantity of both selected species of mold within building wall materials. Real-time qPCR reactions were run in 96 well PCR plates with Microseal® 'B' Film with an iQ™5 Real-Time PCR Detection System (Bio-Rad, Hercules, CA). Each 20µl reaction contained 10µl 2x iQ™ SYBR® Green Supermix (Bio-Rad, Hercules, CA), 1µl each forward and reverse (20µM) species- specific primers. The species-specific primers were for *A. fumigatus* forward primer (5'GCCCGCCGTTTCGAC-3') and reverse primer (5'CCGTTGTTGAAAGTTTTAACTGATTAC-3') and *S. chartarum* forward primer (5' TCCCAAACCCTTATGTGAACC-3') and reverse primer (5' GTTTGCCACTCAGAGAATACTGAAA-3'). These primers were designed by EPA scientists and listed on the following website: http://www.epa.gov/microbes/moldtech.htm. and are designed from the ITS1 region of each species. (Haugland et al 1999;Haugland et al 2002). In addition to the reagents, either 4µl of sample DNA was added to each well or 1µl of sample extract after freeze drying. The reaction protocol included an initial denaturation at 95°C for 2 minutes, followed by 45 cycles of a 15 second 95°C denaturation, 30 second annealing at 60°C, and a 30 second 72°C extension. Extracted fungal genomic DNA was used to develop a standard curve for each species (Figure 2.1 and 2.2). Threshold cycle values and

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concentrations were determined by the Bio-Rad IQ software. After comparing C_T and concentration values from the standard curve value ranges, any values that showed poor amplifications were listed as below detection limit. If the C_T values were above standard curve value, the sample were diluted and re-analyzed for detection. Each C_T value that was within the standard curve values were converted to nanograms of genomic DNA per milligram of each material. The results of each conversion were then averaged for each set of treatments. Figure 2.1 and 2.2 shows the standard curve used for each species.

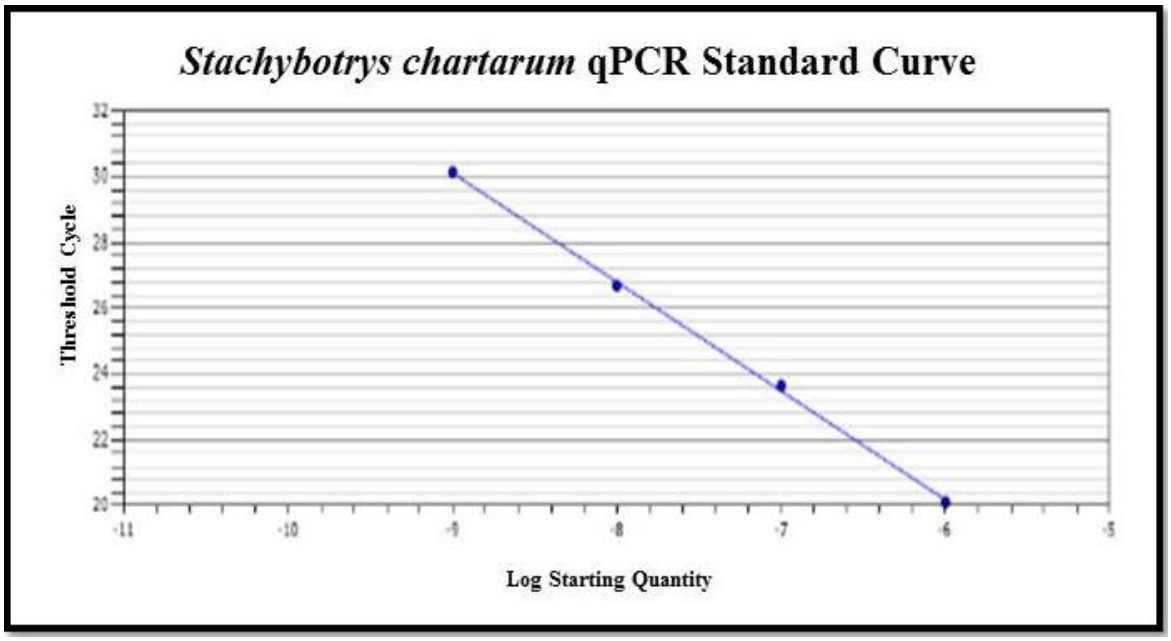

Figure 2.1 Quantitative PCR standard curve of *Stachybotrys chartarum*.

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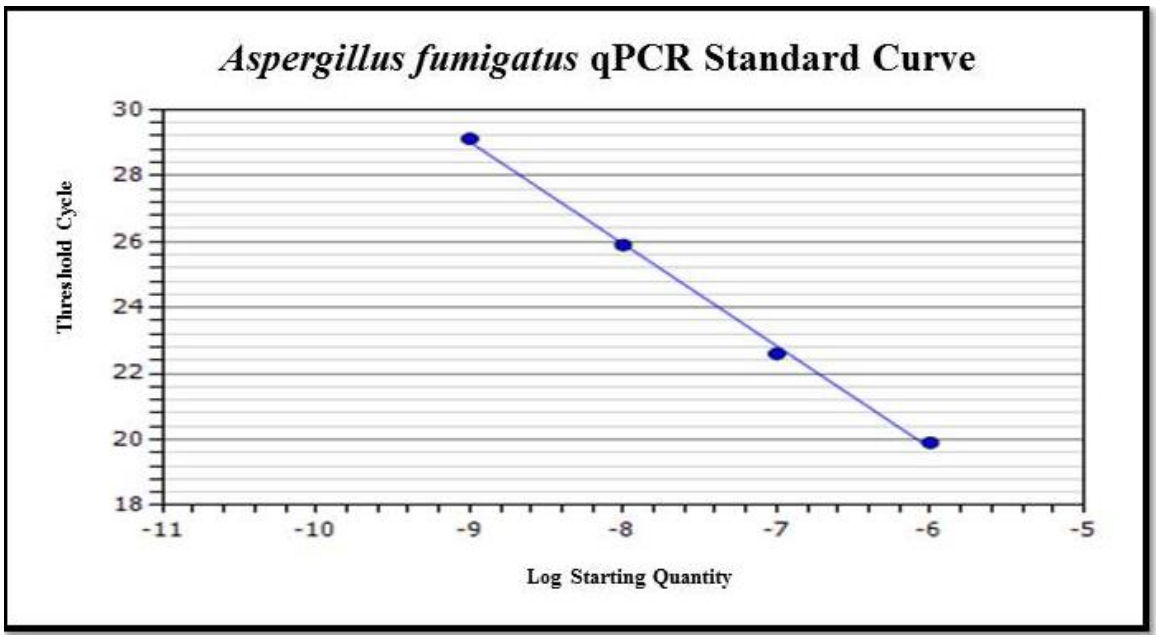

Figure 2.2 Quantitative PCR standard curve of *Aspergillus fumigatus*.

RNA Extraction

RNA extraction was performed using the Ambion RNAqueous Total RNA Isolation Kit (Ambion, Austin, TX) with an added DNase I (Ambion, Austin, TX) digestion step to remove unwanted genomic DNA contaminants. For each sample, five tubes containing 0.2g of OSB and plywood sheathing and 0.1g of fiberglass batt insulation and cellulose batt insulation were placed on ice and 1mL lysis solution (denaturation buffer) added. Tubes were homogenized using a bead mill for three minutes, incubated on ice for three minutes, then homogenized again for three minutes. After homogenization, tubes were centrifuged at 10,000 RCF for two minutes and RNA extraction protocol was completed. In a new tube, DNase I digestion was prepared by adding 42µl DNase-sterile water, 5µl DNase I buffer, and 3µl DNase I enzyme. The digestion mixture was mixed and 50µl was added directly to each filter and incubated at

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room temperature for 15 minutes before proceeding to the next protocol step. After an centrifugation step, the RNA was eluted and was kept on ice until stored at -70ºC. RNA was quantified by a NanoDrop 1000 spectrophotometer (Thermo Scientific, Waltham, MA), and quality was determined by Experion chip electrophoresis (RNA StdSens Analysis Kit, Bio-Rad; Hercules, CA).

cDNA Synthesis

RNA was synthesized to first strand cDNA by the Invitrogen Superscript II Reverse Transcriptase Kit (Carlsbad, CA). For each sample, 2µl of random hexamer primer (Promega, Madison, WI), 2µl of 10mM dNTP mix, and 20µl of RNA template (1µg) and sterile water were added to a 0.2 mL tube and heated for 5 minutes at 65ºC then immediately returned to ice. Four µl of 5x First-Strand Buffer, 2µl of 0.1 M DTT, and 1µ RNaseOUT was added to the reaction mix and incubated at 25ºC for 10 minutes. After incubation, 1µl (200 units) of Superscript II RT was added to each reaction and mixed by pipetting gently up and down. cDNA synthesis was carried out using an Eppendorff Mastercycler at the settings: incubation at 25°C for 10 minutes, incubation at 42°C for 90 minutes, and inactivation of the reaction by heating to 85°C for 5 minutes.

Gene Expression

To determine gene expression, Reverse Transcriptase Quantitative Polymerase Chain Reaction (RT-qPCR) was monitored in real time for the genes selected. RT-qPCR reactions were carried out in an iCyclerIQ™ 96 well PCR plates with Microseal® 'B' Film with an iQ™5 Real-Time PCR Detection System (Bio-Rad, Hercules, CA). The analyses for genes of interest were performed on one plate and the samples that showed

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good RNA quality were measured in triplicate. Each 20µl reaction contained 10µl 2x iQ™ SYBR® Green Supermix (Bio-Rad, Hercules, CA), 6µl of sterile molecular grade water, 1µl of gliotoxin gene gliP forward primer (5' TCCAACAGTCAGAGGCATTC 3') and gliP gene reverse primer (5' CTTGAGGGATAATCGGTGGT 3') (10µM) as described by (Gravelat et al. 2008) expressed by the species *Aspergillus fumigatus*, or 1µl of the TRI5 gene, forward primer (5' GCACACTGGACTGTAAGTCT 3') and reverse primer (5' CCTGGGAAGCCATGGAAGT 3') listed by Black et al. (2008) expressed by *Stachybotrys chartarum*, and 2µl cDNA template. The reaction protocol included an initial denaturation at 95°C for 3 minutes, followed by 45 cycles of a 15 second 95°C denaturation, 30 second annealing at 60°C, and a 30 second 72°C extension. A melt curve analysis was done immediately after amplification which consisted of a 1 minute incubation at 95°C followed by 1 minute annealing at 60°C prior to 81 cycles of 15 seconds at 55°C with temperatures increase 0.5°C each cycle. Following amplification, threshold (C_T) for each sample was calculated and gene expression data values were normalized against expression data for the 18S Housekeeping gene for *Aspergillus fumigatus* forward primer (5'GGCCCTTAAATAGCCCGGT 3') and reverse primer (5' TGAGCCGATAGTCCCCCTAA 3') as listed by Gravelat et al. (2008) and for *Stachybotrys chartarum* forward primer (5' TACAAGCCCTGACTTCGGAA 3') and reverse primer (5' CATGTTTGGCCACTACCCAA 3') as described by Black et al. (2008) using the $2^{-\Delta\Delta C_T}$ method. The same procedures were used in phase II, however the only gene that was selected was TRI5 produced by *S. chartarum*.

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Enzyme-Linked Immunosorbent Assay (ELISA)

Detection of trichothecenes was accomplished using an EnviroLogix QuantiTox Kit (Portland, ME) by preparing a 96-well plate designed for quantitative laboratory analysis detection. Each material sample replicate was placed in a 15mL tube with 10mL of phosphate buffered saline (PBS) Extraction/Dilution Buffer pH 7.4 $\pm$ 0.05 and mixed on an orbital shaker for ten minutes at 200 rpm. After shaking for ten minutes, 1mL of supernatant from each sample was placed into 1.5mL tubes and centrifuged for five minutes at 10,000 rpm. A 900ppb (parts per billion) Roridin A stock solution, which was provided by the kit, was prepared as directed and diluted to 18.0 ppb, 2.0ppb, and 0.2 ppb in PBS buffer. The negative calibrator used was PBS. The limit of detection for this assay is 0.14ppb. Once all components reached room temperature, 50µl of negative calibrator, 50µl of Roridan A calibrator, and 50µl of each sample extract was added to their designated wells. 50µl of enzyme conjugate was immediately added to each well. The plate was then rapidly moved in a circular motion on the bench top for 30-45 seconds following protocol. The plate was then covered with Microseal® 'B' Film and incubated at ambient temperature for 45 minutes using an orbital shaker at 200rpm. After incubation the plate was flooded with distilled water and emptied. This wash step was repeated four more times and inverted several times on a paper towel to remove as much water as possible. After drying, 100µl of substrate was added to each well and thoroughly mixed. Finally 100µl of stop solution was added and mixed thoroughly following EnviroLogix protocol. Spectrophotometric measurement analysis was performed using an Epoch Microplate Spectrophotometer (BioTek, Winooski, VT) set to a wavelength to 450 nanometers and a standard curve was assessed using Roridan A calibrators. The lowest

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detection limit of the ELISA kit is 0.14ppb. Non-inoculated materials were also processed to determine if there was any background interference.

Thin Layer Chromotography

Samples that were inoculated with *A. fumigatus* were analyzed for gliotoxin production using Thin Layer Chromotography (TLC). Each sample material was placed into vials and suspended with 20mL of methanol. Then each sample material was air dried with compressed air. Once dried, the vials were placed into -70°C. Pure gliotoxin (5mg) was suspended in 10mL of methanol for a final concentration of 0.5mg/ml that would be used as a standard. The protocol used 20 x 20cm TLC Silica Gel $60F_{254}$ (Merk, Darmstadt, Germany) marked with 2cm from the bottom with 1cm increments for each material sample. Two eluent systems including toluene: ethyl acetate: formic acid (5:4:1, vol/vol/vol) and chloroform: acetone: iso-propanal (85:15:20, vol/vol/vol) were initially assessed for detection of gliotoxin band formation (Samson et al. 2010). Toluene: ethyl acetate: formic acid resulted in better separation of gliotoxin standard and therefore was used for testing of material samples. 2mL of methanol was placed into each vial, mixed and then 20µl was pipetted onto each TLC plate for each sample and standard until dried and repeated with another 20µl until dried for a total of 40µl for each sample and standard. Thus 0.01mg of standard was visualized on each plate. The TLC plate was then placed into the holding tank with 1.0cm of eluent and the solvent front migrated for approximately 15 to 25 minutes. The TLC plates were air dried in a fume hood for at least 20 minutes and then examined under long wave UV-light and short wave UV- light. It was determined that using the short wave ultraviolet light at 254 nm using the Spectroline Model ENF-24 (Westbury, New York) allowed for a better visualization of

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band formation for gliotoxin as compared to the long wave UV-light at 365nm. Non-inoculated materials were also processed to determine if there was any background interference.

Scanning Electron Microscopy (SEM)

Selected samples of both the OSB and plywood sheathing were first freeze dried for an hour and transported to the SEM facility for observation of mold species present. The wood samples underwent a staging process with double sided carbon tape to mount the wood pieces onto the stub. After mounting on the stub the wood samples were then coated with 45nm platinum. Samples were viewed on a Zeiss EVO 50 Scanning Electron Microscope at 10 kilovolts (kv).

Experimental study: Building Wall Study

Construction and Inoculation

The interior building wall components (fiberglass batt insulation, oriented strand board, gypsum wallboard, and wood stud) were combined with sheetrock and exterior screws to simulate a residential building wall. Figure 2.3, 2.4, and 2.5 shows respectively, the building wall from the interior gypsum wallboard, the interior OSB panel, and the batt insulation wedged between. Each building wall was approximately 6 inches x 6 inches (15.24cm x 15.24cm) and heat treated for one hour at 100°C before processing. A paver stone was used to resemble a concrete foundation and was also heat treated for one hour and at the same temperature as the walls. The mold chamber was washed and disinfected with 70% alcohol before use. Each wall was then placed onto a heat treated concrete

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paver stone inside the mold chamber. Figure 2.6 shows the concrete paver at the bottom of the mold chamber and Figure 2.7 shows the wall sitting on the paver in the chamber.

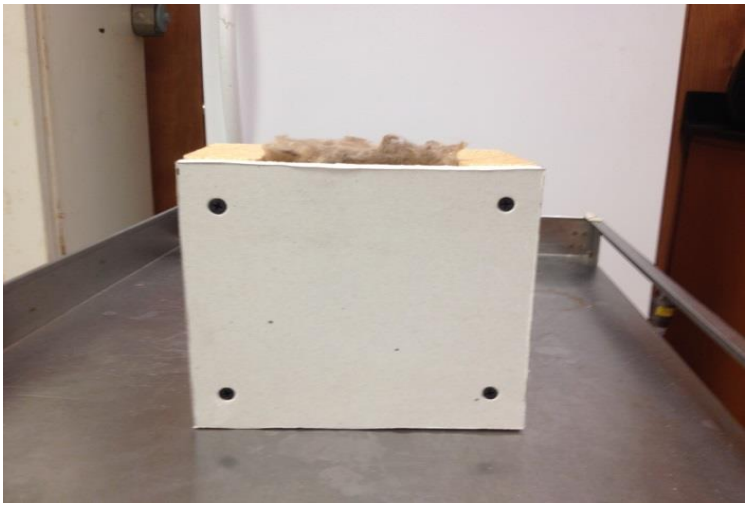

Figure 2.3 Picture of the interior side of the residential building wall showing the gypsum wallboard.

Figure 2.4 The back side of the exterior building wall showing the OSB.

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Figure 2.5 Picture of the inside of the residential building wall showing the wood studs and batt insulation.

Figure 2.6 Paver stone at the bottom of the mold chamber

The wall sat on top of the paver stone during the study.

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Figure 2.7 Residential wall placed into container

Wall sits on a paver stone.

Each chamber containing the paver stone and wall received a total of 4 liters of autoclaved pond water and was left for three weeks at a temperature of 30°C. The chambers were sealed with air tight lids containing 0.2nm filters. The filters allowed for air exchange within the mold chamber but would not allow the mold spores to escape. There were three walls for each collection day. Figure 2.7 shows the mold chamber that was used during the study. The 4 liters of water reached three inches from the top of the wall. The lid of the chamber sits almost flush with the top of the wall. There were three replicate wall units for each collection day. Samples codes are listed in Appendix 1, Table A.2.

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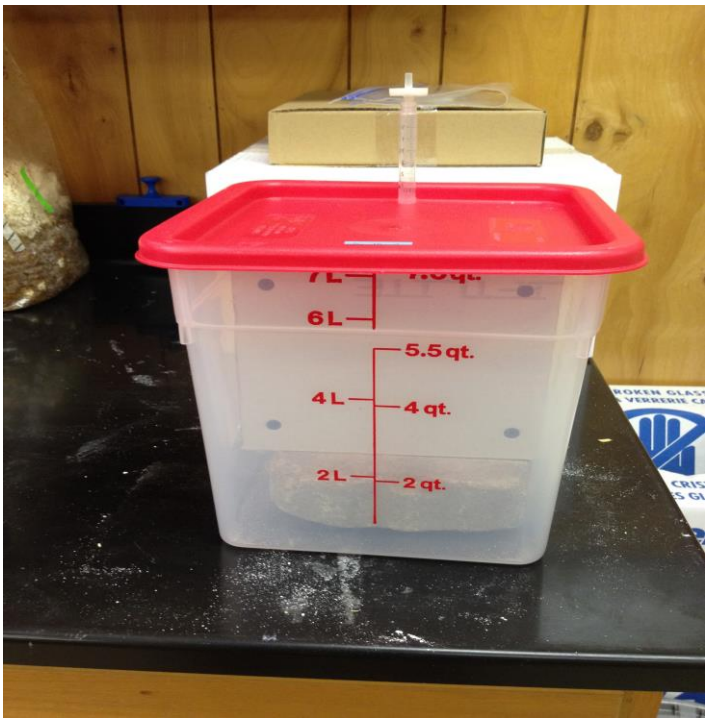

Figure 2.8 The mold chamber with the filter for air exchange and the wall sitting on top of the paver stone.

After three weeks, approximately 2 liters of water were removed from the wall chamber and the walls were inoculated with a suspension of *S. chartarum* ($1.23E^{05}$ cells/ml) using a glass spray unit (Sigma, 50mL) directly onto the wall. The water remaining in the mold chamber was below the top of the paver stone and provided the moisture needed to maintain high humidity. Inoculation suspension was prepared as previously described. Spore concentration was determined using a haemocytometer and approximately 2mL of inoculum were sprayed into each wall. After each selected day (15, 35, 50, and 65) the building wall materials were processed and assessed for both fungal growth and mycotoxin production. First the batt insulation was removed from between the wood studs. Then the gypsum wall board was detached from the wood studs

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and the paper was stripped from the gypsum itself. The wood studs were detached from the oriented strand board so that scraping could occur. All three replicate walls, on selected days, were dismantled. Under cold room conditions (2°C) the wood was scraped on all sides and placed into designated five subsample collection bags per wall. Finally, the gypsum paper and batt insulation backing was stripped and cut with sterile scissors into small stripes and collected into five subsample collection bags per wall.

Sample Preparation

The building materials (batt insulation paper, gypsum wallboard front and back paper, wood stud scrapings, and oriented strand board scrapings) were weighed, placed into 2mL screw capped tubes, and placed into -70 C until processing. Five subsamples of each building material were extracted for DNA, RNA, and mycotoxin analysis following the procedures as described previously. The genomic DNA was analyzed by qPCR using the same procedures and primers and the RNA was analyzed by RT-qPCR for the TRI5 gene following previously described procedures. Mycotoxin analysis was by ELISA as previously described except that only 2mL of buffer was used for extractions. In addition to the interior building materials, the concrete paver stone was swabbed using BBL CultureSwab (Copan, Sparks, MD) and placed at -20°C until processing. The swabs were cut with sterile scissors and placed into 2mL buffer provided by the ELISA kit. The swabs were then processed as described previously and analyzed for trichothecenes.

Statistical Analysis

The genomic DNA samples were converted to nanograms per milligram of material, and statistical differences were determined for the different building materials.

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ANOVA test with Tukey's was used to determine if the means of the different building materials were significantly different using SAS9.4. Statistical differences were also determined for the mycotoxin data as comparison of means among the different building materials for each collection day as well as over time.

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CHAPTER III

RESULTS AND DISCUSSIONS

Preliminary Study: Petri Dish Analysis Results

This preliminary study evaluated the expression and quantitation of both gliotoxin and trichothecenes on different building materials. A total of 160 samples were examined in this study. Eighty samples were inoculated with *A. fumigatus* and eighty inoculated with *S. chartarum*. Materials consisted of fiberglass batt insulation (R-13), cellulose batt insulation, plywood sheathing 1.27cm x 4.919cm x 0.952cm (12.7mm x 49.19mm x 9.52mm), and oriented strand board 1.27cm x 4.919cm x 0.952cm (12.7mm x 49.19mm x 9.52mm). All material samples were randomly mixed after processing; however they were placed into designated bags for each collection day and building material.

DNA Quantification

DNA Quantification was first prepared by scraping both OSB and plywood sheathing when the wood blocks were still saturated. However, once extracted these wood scrapings resulted in very low DNA concentrations as determined by RT-PCR. These results conflicted with the micrographs taken using the Scanning Electron Microscope, which showed an abundance of fungi present on the wood at least for *S. chartarum*. Since the micrographs and Real-Time PCR results were conflicting, a second set of samples were prepared by using a freeze dryer to dry the wood blocks. Scrapings from the freeze-dried wood samples were then extracted and DNA concentrations

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determined by Real-Time PCR. The results from the freeze-dried samples were much more realistic and are shown in Appendix B (Tables B.1 and B.2). There was not enough batt and cellulose insulation to redo the DNA extractions on freeze dried material, thus this resulted in either no mold present or very low DNA concentrations for these materials.

Aspergillus fumigatus

The average concentration of *A. fumigatus* on each building material for each day is shown in Figure 3.1.

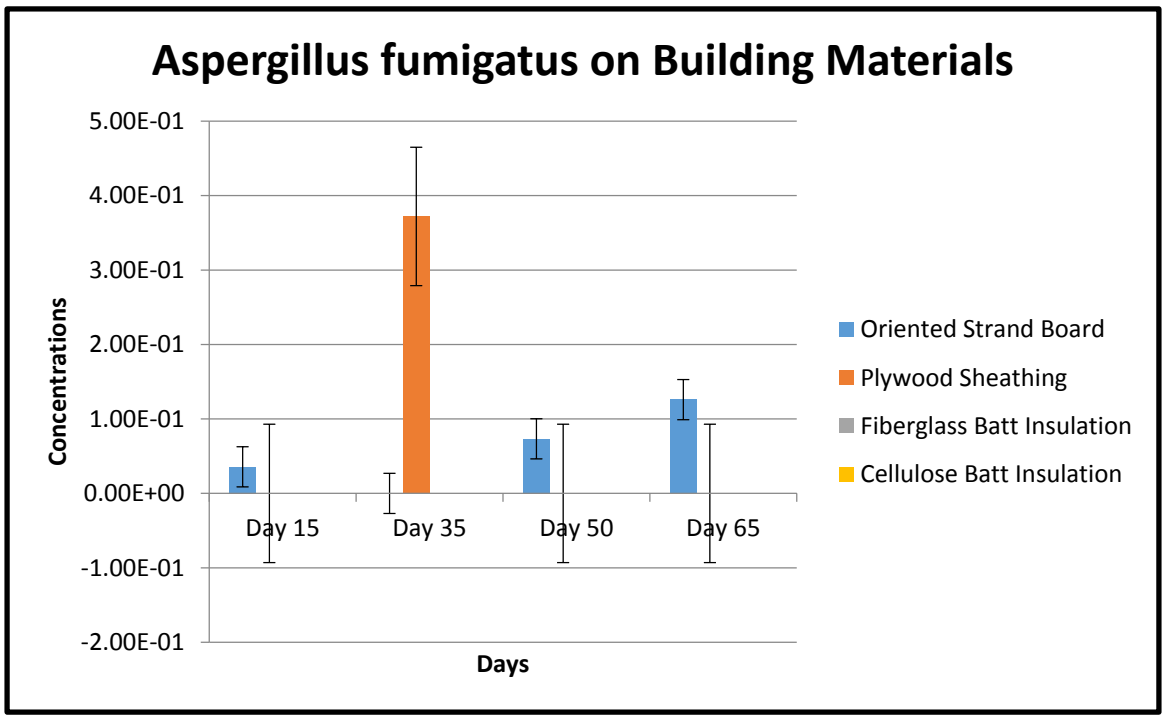

Figure 3.1 *Aspergillus fumigatus* DNA concentrations in nanograms of DNA per milligram of material on different building wall materials.

Error bars represent the standard error of the mean.

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On day 15 the oriented strand board had a concentration of $3.56E^{-02}$ while the plywood sheathing, fiberglass batt insulation, and cellulose batt insulation had much lower DNA concentrations. On day 50 and 65, the only building material that contained notable concentrations of *A. fumigatus* was the oriented strand board. DNA concentrations on OSB increased over time. DNA on plywood sheathing was only detected at a notable concentration at day 35. One disadvantage of qPCR is its extreme sensitivity. There were only three replicates processed for plywood on day 35. One of these replicates contained no detectable level of DNA, while the other two had high concentrations. Since very small amounts of wood are extracted for each sample, a selection of a few pieces containing a lot of fungi could skew the results. A larger number of replicates need to be processed in order to minimize this variation. Statistics were performed for all building materials inoculated with *A. fumigatus* and collection day are presented in Tables 3.1 and 3.2.

Table 3.1 Statistical analysis (P=0.05) of *A. fumigatus* DNA concentrations on the building materials when averaged for each time.

Tukey Grouping	Mean	Samples (N)	Building Material
A	0.0925	4	Plywood Sheathing
A	0.0587	4	Oriented Strand board
A	1.37E-7	4	Cellulose Batt Insulation
A	4E-8	4	Fiberglass Batt Insulation

Means with the same letter are not significantly different.

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Table 3.2 ANOVA (P=0.05) statistical analysis of DNA concentrations of *A. fumigatus* averaged for each collection day on the different building materials.

Tukey Grouping	Mean	N	Day
A	0.0925	4	35
A	0.0315	4	65
A	0.01833	4	50
A	0.0089	4	15

Means with the same letter are not significantly different.

ANOVA analysis shows no statistical difference among the building materials or the collection day. The averages shown in Table 3.2 highlights the high variation among subsamples masking possible significant difference. Figures 3.2 and 3.3 show *A. fumigatus* present on both types of wood for day 65 using SEM. *A. fumigatus* was sparse on plywood but the mycelia heavily covered the OSB.

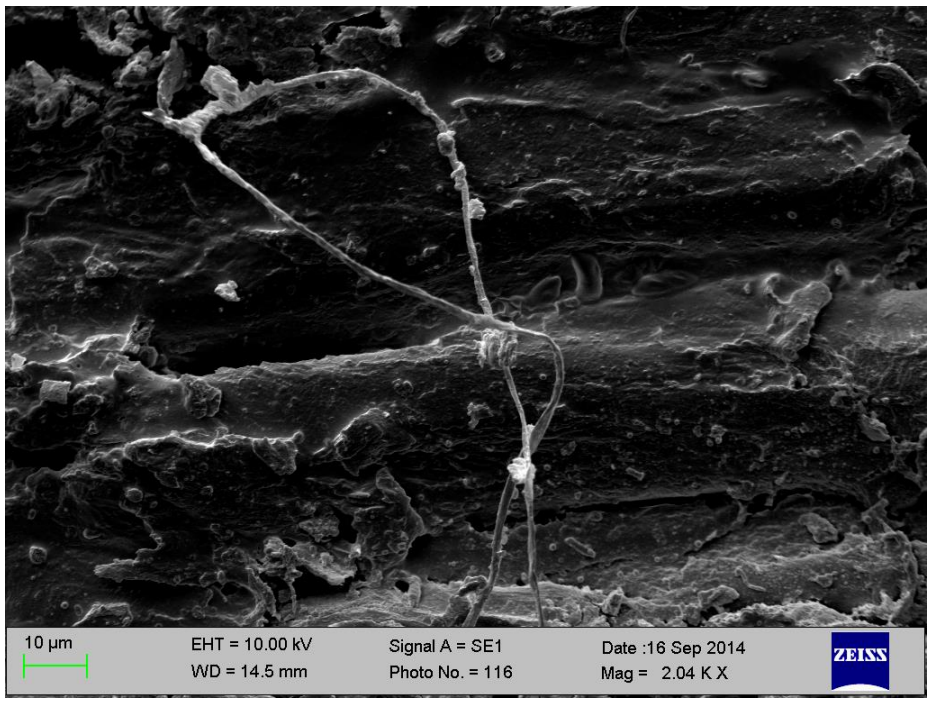

Figure 3.2 Micrograph of *Aspergillus fumigatus* on Day 65 on plywood building material sample.

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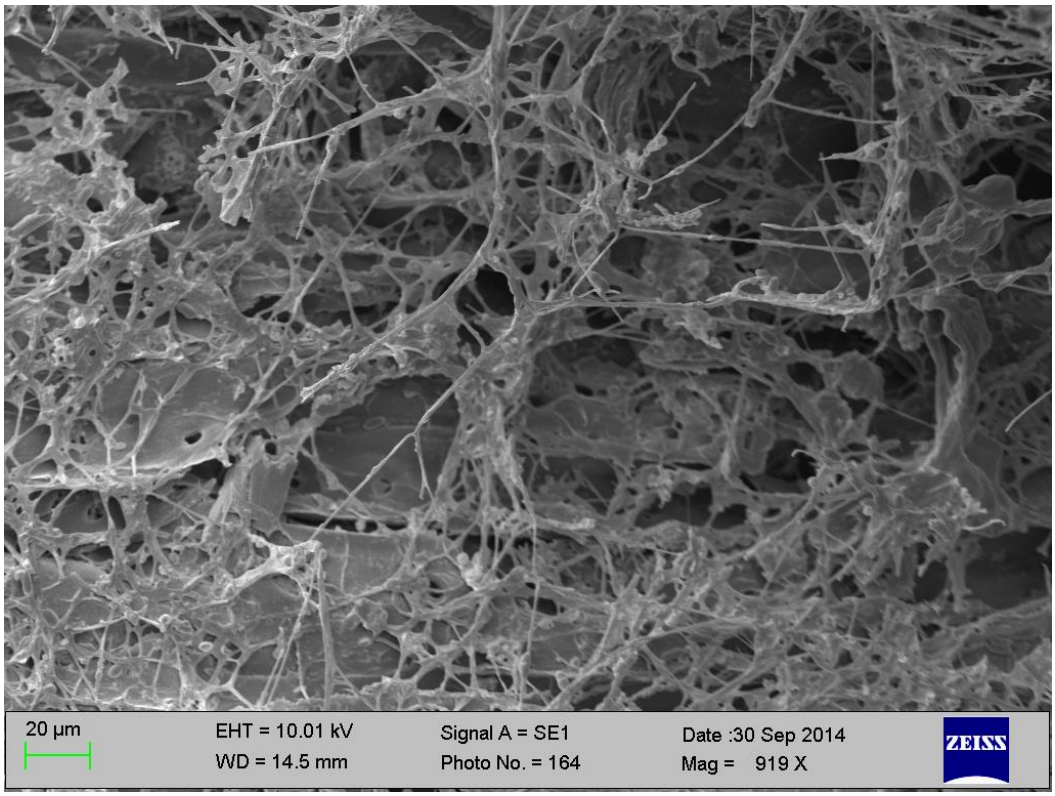

Figure 3.3 Micrograph of *Aspergillus fumigatus* on Day 65 on oriented strand board material samples.

Stachybotrys chartarum

Building material samples were also inoculated with *S. chartarum* and DNA concentrations are shown in Appendix B (Table B.2). The average concentration of *Stachybotrys chartarum* on each type of building material on the selected day is shown in Figure 3.4.

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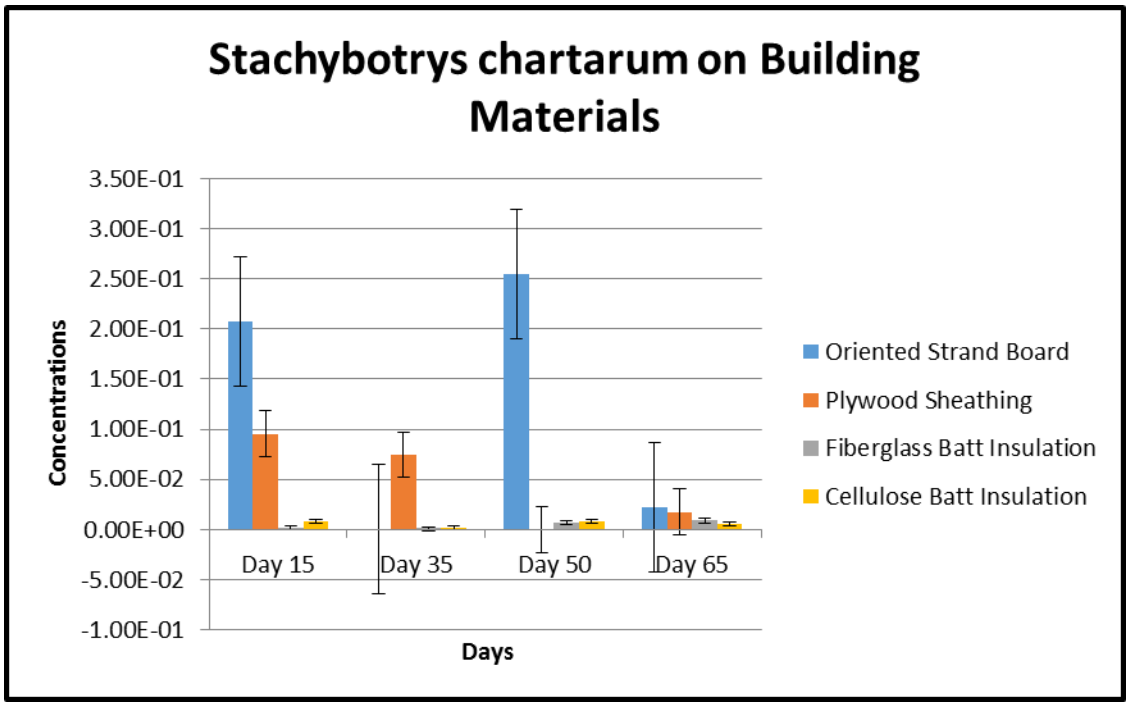

Figure 3.4 The distribution of *Stachybotrys chartarum* DNA concentration in nanograms of DNA per milligram of material on different building wall materials.

Error bars represent the standard error of the mean.

Overall concentrations of *S. chartarum* DNA were high on the oriented strand board as compared to the other building materials (plywood sheathing, fiberglass batt insulation, and cellulose batt insulation). On day 15, the oriented strand board had a DNA concentration of $2.07E^{-01}$ while plywood sheathing had the next highest of $9.55E^{-02}$. It is interesting that cellulose batt insulation supported a concentration of $8.08E^{-03}$, which is likely due to the fact that cellulose can be a nutrient source for mold. On day 35, no *S. chartarum* was detected on the oriented strand board. Day 50 showed the highest DNA concentration of $2.55E^{-01}$ on the oriented strand board while both cellulose and fiberglass batt insulation had low concentrations. Finally, on day 65 all building materials exhibited lower concentrations of *S. chartarum*. The absence of detectable DNA on OSB at day 35,

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once again could be due to the variability in selection of small wood samples. Statistics are given in Table 3.3 and 3.4 for the building materials and collection day.

Table 3.3 Statistical analysis (P=0.05) of DNA concentrations of *S. chartarum* as averaged for all building materials.

Tukey Grouping	Mean	N	Building Material
A	0.12095	4	Oriented Strand board
A	0.04693	4	Plywood Sheathing
A	0.00583	4	Cellulose Batt Insulation
A	0.00437	4	Fiberglass Batt Insulation

Means with the same letter are not significantly different.

Table 3.4 Statistical analysis (P=0.05) of *S. chartarum* concentrations averaged for each collection day.

Tukey Grouping	Mean	N	Day
A	0.07822	4	15
A	0.06739	4	50
A	0.01906	4	35
A	0.0134	4	65

Means with the same letter are not significantly different.

Statistics show no significant difference among the building material or collection day. However, the bars in Figure 3.4 represent the standard error of each building material. Oriented strand board is different from plywood sheathing since the error bars do not overlap on Days 15 and 50. In addition, the oriented strand board and both types of insulations are different on Days 15 and 50. Figures 3.5 and 3.6 show *S. chartarum* presence on oriented strand board on day 65 in SEM micrographs.

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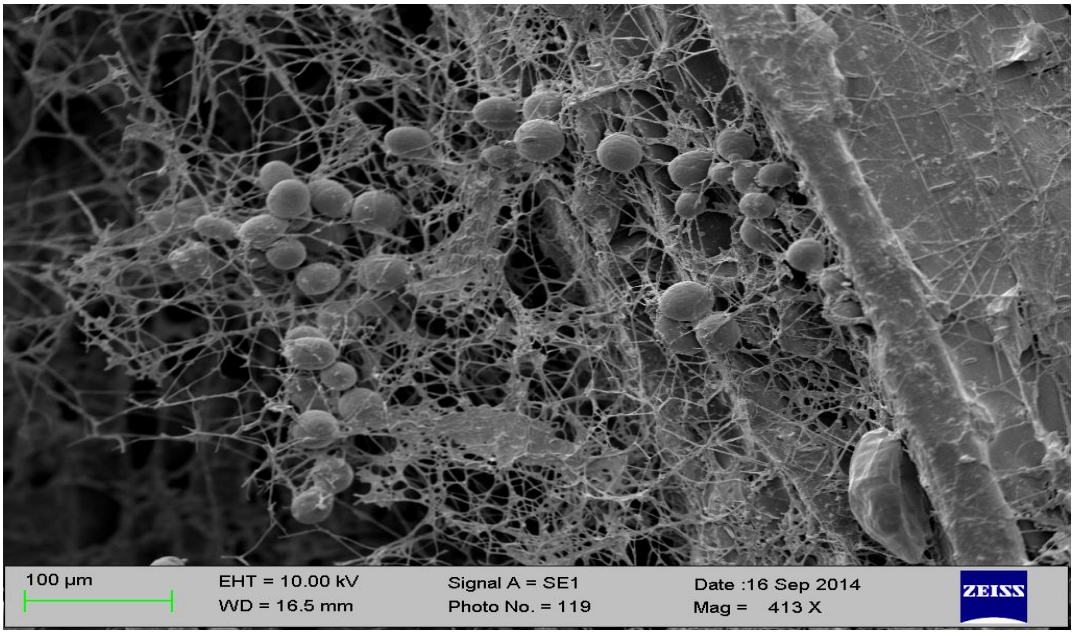

Figure 3.5 Scanning electron microscopy micrograph of *S. chartarum* on oriented strand board on day 65.

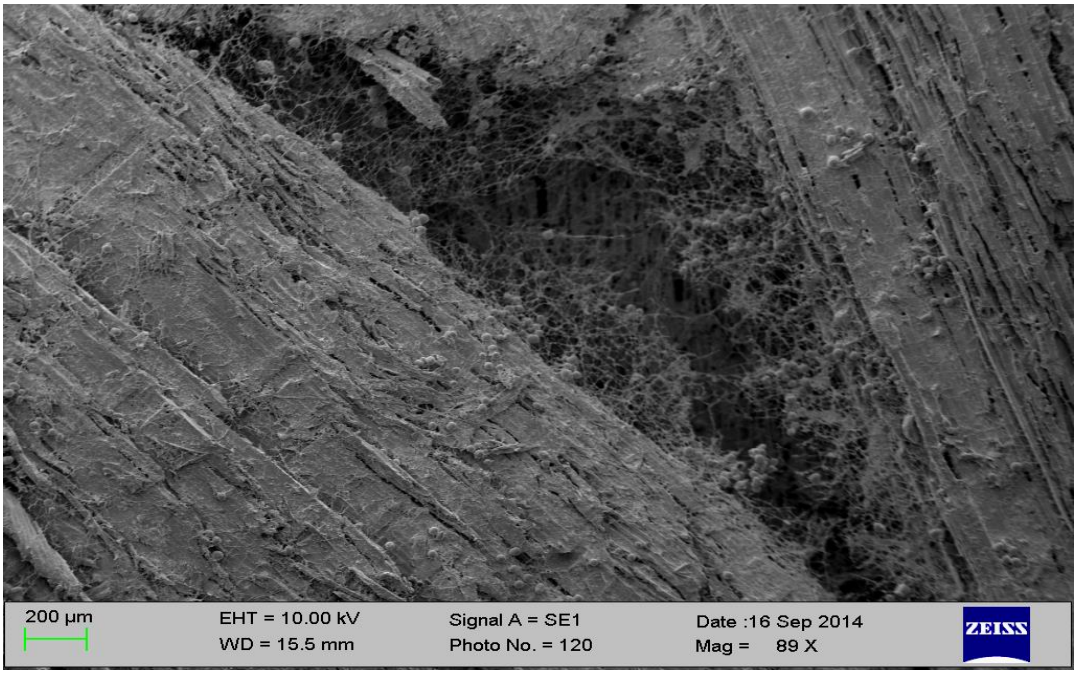

Figure 3.6 Scanning electron microscopy micrograph of *S. chartarum* on oriented strand board on day 65.

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RNA

RNA quality from each building material was assessed using the Experion for both *A. fumigatus* and *S. chartarum*. The Experion indicates RNA quality by color; green for excellent quality, yellow is moderate, and red is poor. The results in Appendix B (Table B.3) show that most of the RNA quality from *A. fumigatus* was poor or extracted in very low concentrations. Moderate results were obtained from oriented strand board as compared to the other building materials. The excellent results were mainly scattered among the different building materials.

Appendix B (Table B.4) shows that most of the RNA quality from *S. chartarum* was also in the poor or very low concentrations range. Once again the moderate results mainly came from the oriented strand board as compared to the other building materials. The excellent results were scattered among the different building materials, however cellulose batt insulation day 35 showed good quality RNA with the exception of two replicates.

Overall RNA was either poor quality or very low concentrations. This could have resulted from the samples being saturated with water and thereby RNA extraction efficiency would have been affected. Also the samples were placed into -70°C for a long period of time and were not extracted in a timely manner. However, the data does show that oriented strand board and plywood sheathing had either a moderate to excellent reading as compared to the other building materials. This shows that sheathing can support mold growth as compared to cellulose batt insulation and fiberglass batt insulation.

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Mycotoxin Detection

Enzyme-Linked Immunosorbent Assay (ELISA)

Since standards could not be found for individual macrocyclic trichothecenes, an Enzyme-linked immunosorbent assay (ELISA) was used to measure trichothecene toxins present on building materials. There are ten types of trichothecenes that this ELISA is able to detect. The trichothecenes include: Satratoxin G&H, Isosatratoxin F, Roridin A,E,H &L-2, Verrucarol, and Verrucarin A&J. All of these mycotoxins have a similar structure in that they all have C-12 and C-13 epoxy ring which allows them to become aerosolized. To estimate concentrations, a standard curve was produced using the Roridin calibrators (18 ppb (parts per billion), 2 ppb, 0.2 ppb, and 0 ppb), which produced a linear equation ($y = -0.0573x + 1.5429$). Optical Density (OD) readings for standards were 0.54 OD for 18ppb, 1.12 for 2ppb, 1.81 for 0.2ppb, and 2.20 for 0ppb. Unfortunately, day 15 samples were not prepared correctly and therefore could not be analyzed by ELISA. Each sample replicate was calculated and averaged for a concentration value and these results are shown in Appendix B (Table B.5).

Figure 3.7 shows the averages for ELISA detection on each day and building material. Cellulose batt insulation supported the production of trichothecenes as compared to fiberglass batt insulation in which there was no detection of the mycotoxin. On the cellulose batt insulation the highest concentration of mycotoxin was on Day 35, Day 50, and Day 65. The plywood sheathing supported a concentration of 2.97 ppb on Day 65 and oriented strand board a very low concentration of 0.85ppb also on Day 65. It is interesting that no mycotoxin was detected on Days 35 and 50 on both the plywood sheathing and oriented strand board. The standard error bars show a difference between

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the cellulose batt insulation and plywood sheathing and oriented strand board for both day 35 and 50. There was no difference between cellulose batt insulation and both plywood sheathing and oriented strand board on day 65.

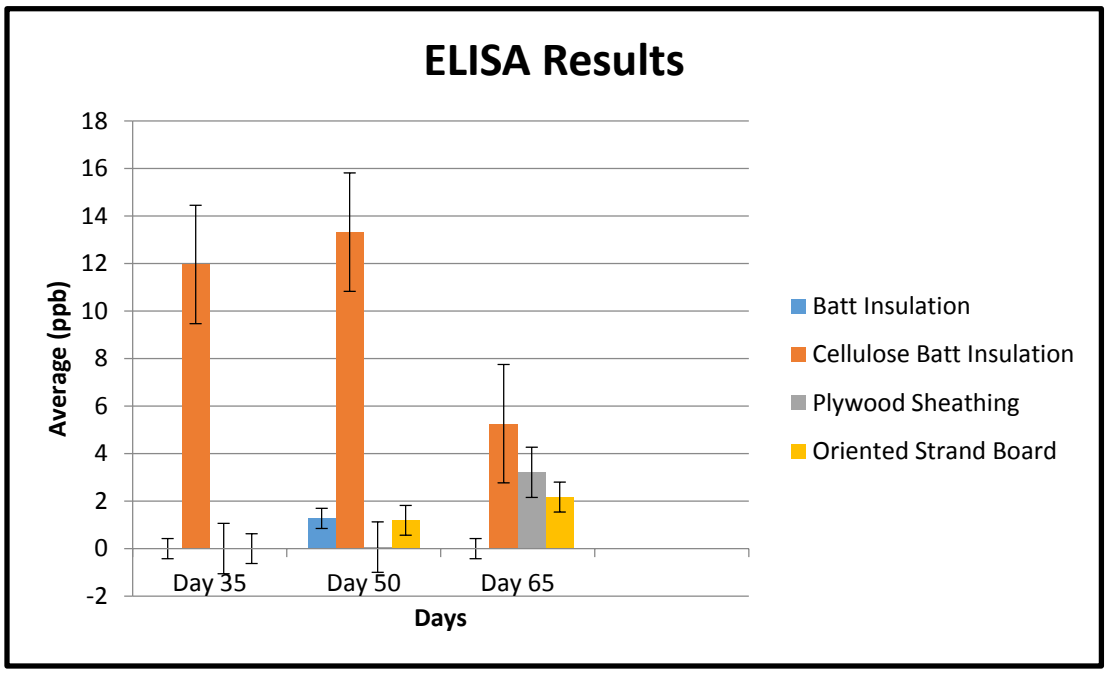

Figure 3.7 ELISA results for trichothecene detection with standard error bars. Error bars represent the standard error of the mean.

The only building material that had supported a high concentration of trichothecenes was the cellulose batt insulation. There were detectable mycotoxin levels on the fiberglass batt insulation and oriented strand board on day 50 and the plywood sheathing and oriented strand board on day 65.

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Thin Layer Chromatography

Samples that were inoculated with *A. fumigatus* were analyzed for gliotoxin production by Thin Layer Chromatography (TLC). Results for each sample material and replicates for gliotoxin are shown in Table 3.5.

Table 3.5 Thin Layer Chromatography results of gliotoxin present on different building wall materials.

Day	OSB	Plywood Sheathing	Cellulose Batt Insulation	Fiberglass Batt Insulation
15	2/5	0/5	0/5	0/5
35	0/5	2/5	0/5	0/5
50	1/5	1/5	0/5	0/5
65	1/5	0/5	0/5	0/5

Number with a positive band for gliotoxin/number of replicates tested.

Both plywood sheathing and oriented strand board showed some presence of gliotoxin. Gliotoxin was present on two of the samples on day 15 and only one sample on day 50 and 65. On plywood sheathing only two samples indicated gliotoxin present at day 35 and only one sample on day 50. The other building materials, cellulose batt insulation and fiberglass batt insulation, showed no signs of gliotoxin being present. Non-inoculated materials were also processed and showed only the gliotoxin standard with no other band formation.

Gene Expression

Gene expression was successful for *A. fumigatus* gliotoxin. Specific primers were used to determine if gliP (gliotoxin) was expressed using cDNA from good quality RNA samples. Figure 3.8 shows the gene expression level of gliP on each building material and day.

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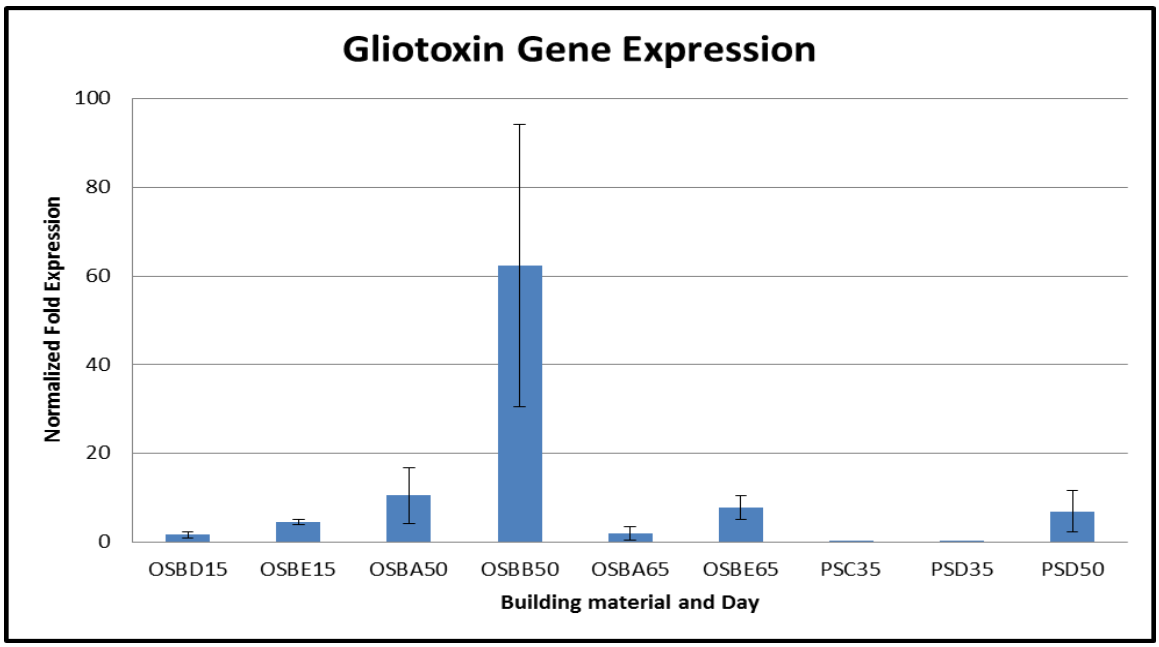

Figure 3.8 Gene expression on selected building material and day amended with the 18S housekeeping gene.

The gene expression of gliP (for gliotoxin) was only conducted on oriented strand board and plywood sheathing (PS). The other building materials either had low concentrations of RNA or very poor quality and therefore could not be converted to cDNA. Most of the oriented strand board samples had low expression of the gliotoxin gene; however it is worth noting that oriented strand board and plywood sheathing on day 50 had their highest expression of the gliotoxin gene as compared to the other sample dates.

Unfortunately it was determined that the gene TRI5 produced by *S. chartarum* was not detected using specific primers and therefore no expression graph was produced. Most of the samples that were converted from RNA to cDNA were mainly of oriented

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strand board and not cellulose batt insulation where it was shown by using an ELISA that trichothecenes were present.

Experimental Study: Building Wall Analysis Results

Figures 3.9-3.11 show the walls dismantled while processing each building wall material for the study.

Figure 3.9 Picture of dismantled wall on day 65.

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Figure 3.10 Picture of gypsum wallboard surface layer on extraction day 65

Figure 3.11 Picture of gypsum wallboard bottom brown paper (side facing the insulation) side on extraction day 65

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The complete data for DNA analysis is given in Appendix B (Table B.6). Figure 3.12 shows the average DNA concentrations of *S.chartarum* on each building material for each day.

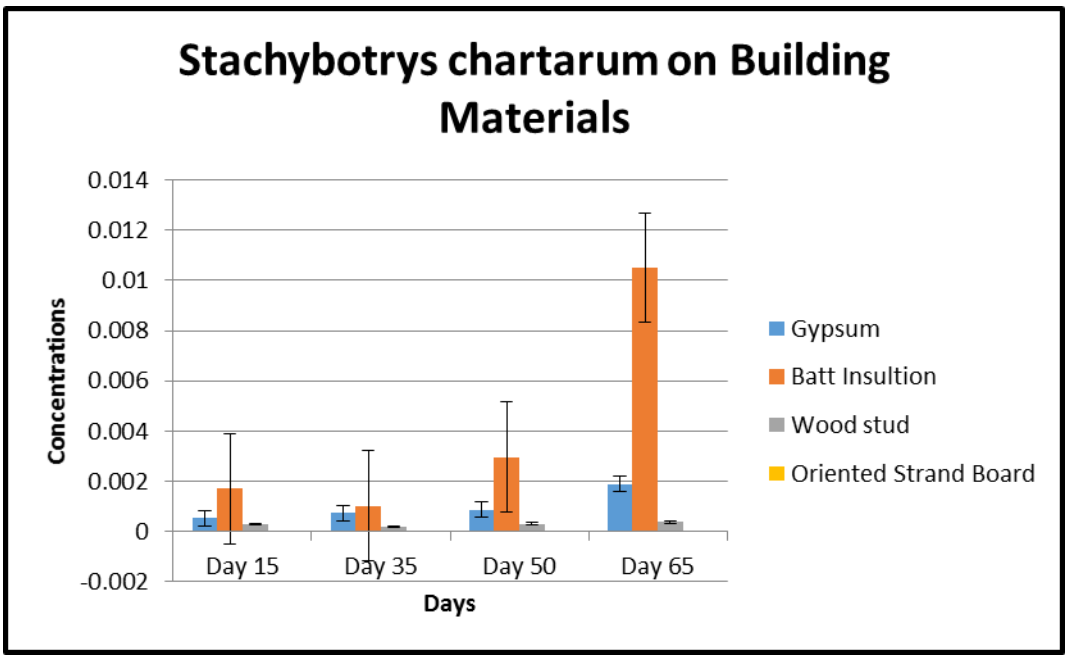

Figure 3.12 *Stachybotrys chartarum* concentrations on building wall materials in nanograms of DNA per milligram of material on different building materials.

Error bars represent the standard error of the mean

Overall DNA concentrations of *S. chartarum* were high on the batt insulation as compared to the other building materials (gypsum wallboard, wood stud, and oriented strand board). On day 15, the batt insulation contained a DNA concentration of $5.11E^{-03}$ while gypsum wallboard had the next highest of $7.32E^{-06}$. The concentration of mold on the fiberglass batt insulation and gypsum increased from day 35 to day 65. On day 15, the lowest concentration detected was on the wood stud, while the oriented strand board contained no detectable concentrations. Day 65 showed the highest concentration of mold

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on the gypsum wallboard and fiberglass batt insulation. Mold concentration on the wood stud and oriented strand board stayed relatively constant throughout the duration of the study. The concentration of *S. chartarum* on the fiberglass batt insulation was statistically greater than the wood stud and OSB (Table 3.6). However, there was no detectable significant difference when comparing collection dates (Table 3.7)

Table 3.6 Analysis of the significant difference (P=0.05) of *S. chartarum* concentrations averaged on different building materials.

Tukey Grouping		Mean	N	Material
	A	0.00725	12	Fiberglass Insulation
B	A	0.00101	12	Gypsum wallboard
B		0.00031	12	Wood Stud
B		0.00001	12	Oriented Strand board

Means with the same letter are not significantly different.

Table 3.7 Statistical analysis (ANOVA) (P=0.05) averaged for each collection day of *S. chartarum* on the different building materials.

Tukey Grouping	Mean	N	Day
A	0.00609	12	65
A	0.00085	12	35
A	0.00082	12	15
A	0.00082	12	50

Means with the same letter are not significantly different.

Each material underwent the same procedure as in the preliminary results and the RNA quality was still poor. Appendix B Table B.7 shows the Experion results for RNA quality of each building materials.

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It was thought that the poor extraction in the preliminary study was due to saturation. However these samples were moist but not saturated. Also these samples were not held in the freezer for very long before extraction. Thus the reason for the poor quality is not known.

Appendix B (Table B.8) and Figure 3.13 show that trichothecenes were detected by ELISA on the building materials throughout all days tested.

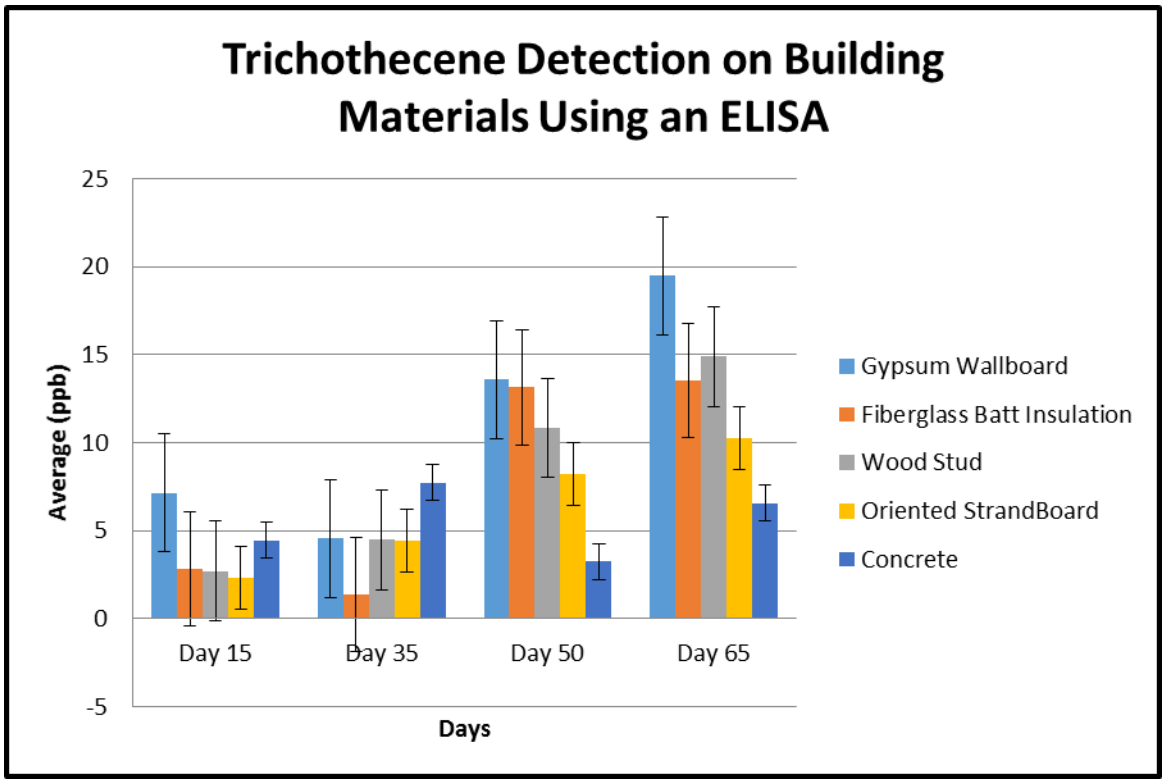

Figure 3.13 Mycotoxin concentration in ppb on different building materials as measured by ELISA.

Error bars represent the standard error of the mean.

Overall the ELISA shows that all building materials contained some level of trichothecenes on every day tested. There were major increases of trichothecene detected

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on both day 50 and day 65. The variation of samples is presented in Figures 3.14 and Table 3.15.

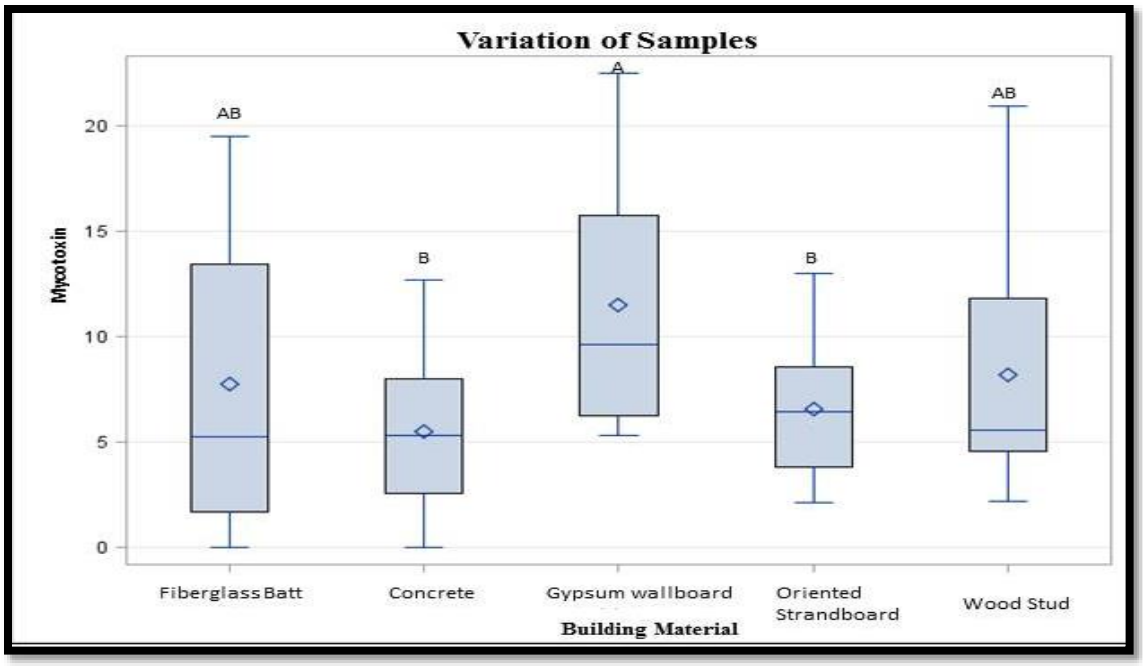

Figure 3.14 ANOVA (P=0.05) analysis of the significant difference of each building material averaged for all collection dates.

Means with the same letter are not significantly different.

Table 3.8 ANOVA (P=0.05) analysis of the significant difference of building wall materials averaged over each collection day.

Tukey Grouping		Mean	N	Material
	A	11.543	12	Gypsum
B	A	8.173	12	Wood stud
B	A	7.784	12	Fiberglass Batt Insulation
B		6.565	12	Oriented Strand board
B		5.498	12	Concrete

Means with the same letter are not significantly different

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The statistical analysis of trichothecenes concentrations shows a significant difference between gypsum wallboard and both oriented strand board and concrete. There was no significant difference between wood stud, batt insulation, and gypsum wallboard. Figure 3.15 presents the distribution of samples and Table 3.9 shows the ANOVA when comparing each collection day averaged for all materials.

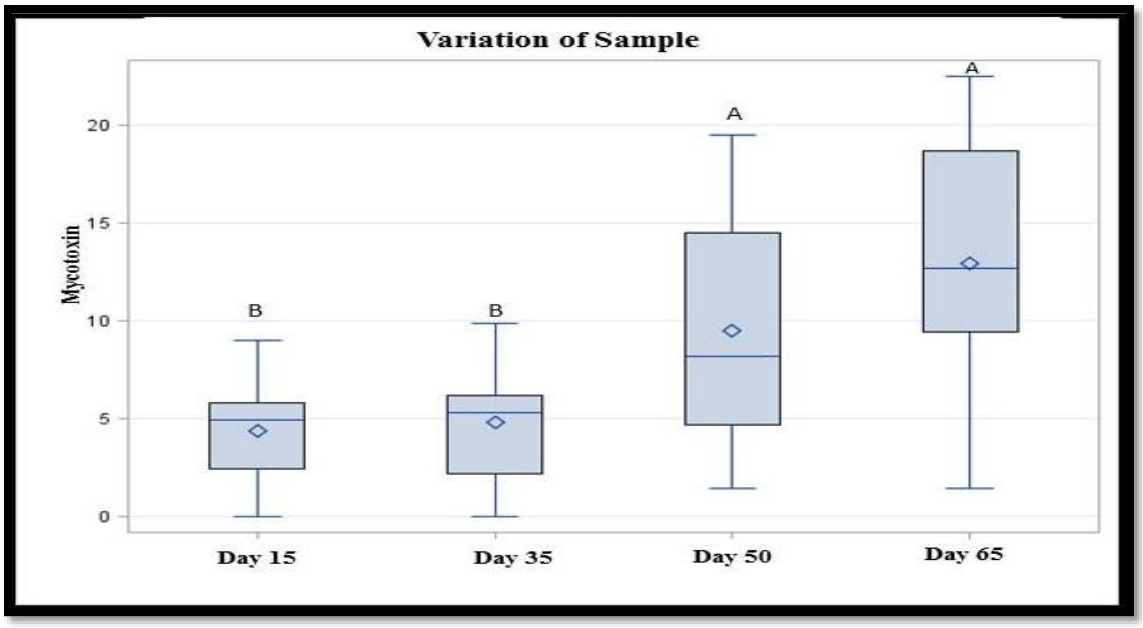

Figure 3.15 Statistical (ANOVA) (P=0.05) analysis of the significant difference of trichothecene concentration on each day averaged for all materials.

Means with the same letter are not significantly different.

Table 3.9 ANOVA (P=0.05) analysis of the significant difference of each collection day averaged for all materials.

Tukey Grouping	Mean	N	Day
A	12.937	15	65
A	9.506	15	50
B	4.834	15	35
B	4.375	15	15

Means with the same letter are not significantly different.

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The ANOVA (Table 3.10) shows that Day 50 and Day 65 are significantly greater than Day 15 and Day 35. Table 3.10 presents the statistical analysis of each building material for each experimental day.

Table 3.10 Statistical analysis (ANOVA) (P=0.05) of the average detection of trichothecene concentration on different building wall materials and collection day.

Tukey Grouping		Mean	Sample (N)	Building Material and Day
	A	19.485	3	Gypsum (65)
B	A	14.891	3	Wood Stud (65)
B	A C	13.576	3	Gypsum (50)
B	A C	13.52	3	Batt Insulation (65)
B	A C	13.139	3	Batt Insulation (50)
B	D A C	10.231	3	Oriented strandboard (65)
B	D A C	9.349	3	Wood Stud (50)
B	D C	8.221	3	Oriented Strandboard (50)
B	D C	7.73	3	Concrete (35)
B	D C	7.2	3	Gypsum (15)
B	D C	6.557	3	Concrete (65)
B	D C	5.91	3	Gypsum (35)
B	D C	4.465	3	Wood Stud (35)
B	D C	4.463	3	Concrete (15)
B	D C	4.425	3	Oriented Strandboard (35)
B	D C	3.988	3	Wood Stud (15)
	D C	3.384	3	Oriented Strandboard (15)
	D C	3.243	3	Concrete (50)
	D C	2.841	3	Batt Insulation (15)
	D	1.638	3	Batt Insulation (35)

Means with the same letter are not significantly different.

In Table 3.10, gypsum wallboard on day 65 is significantly greater than gypsum wallboard on day 15 and day 35 but not on day 50. In addition gypsum wallboard actually increased in mycotoxin detection from day 15 to day 65. Trichothecene on

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fiberglass batt insulation on day 35 was significantly lower in mycotoxin levels compared to day 50 and 65. The wood stud, oriented strand board, and concrete were not significantly different on any collection day.

The concrete results are presented separately in Appendix B (Table B.9) and Figure 3.16 where additional samples were taken on Day 160.

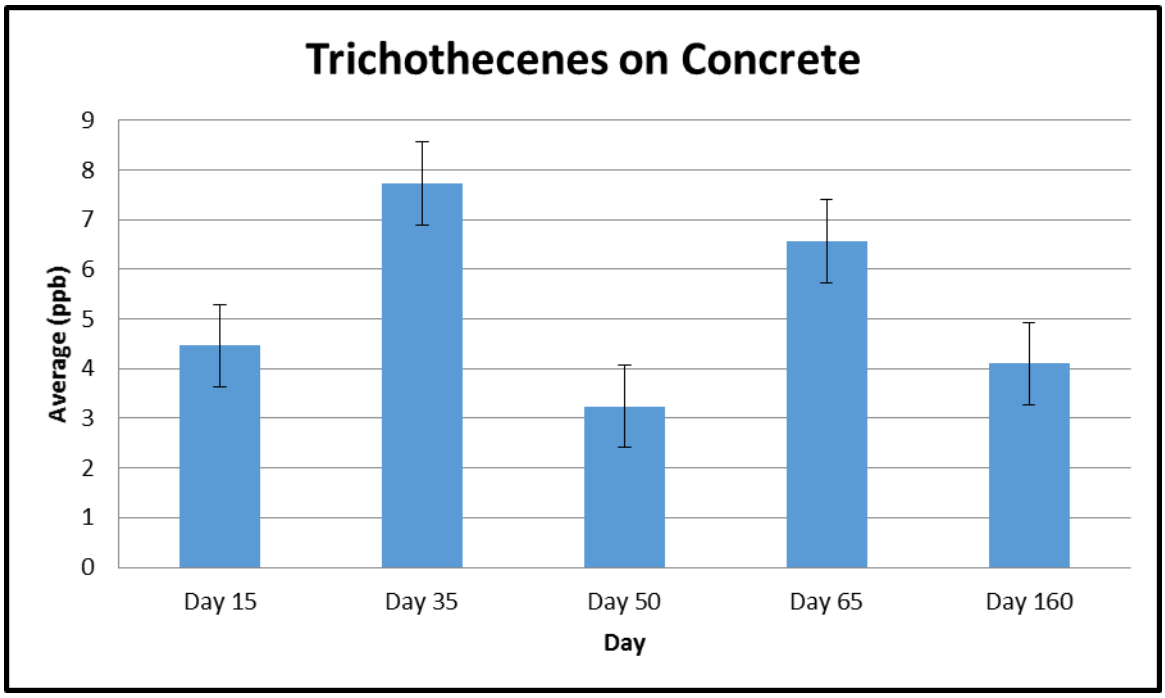

Figure 3.16 Trichothecenes on the concrete slab on different days as determined by ELISA.

Figure 3.16 shows the averages trichothecene concentrations of samples taken from the paver stone believed to be made of concrete and analyzed using ELISA. The most trichothecene was detected on day 35 while the least amount was on day 50. Trichothecenes were detected on paver stone at high levels even at 160 days. However there were no significant differences among the different collection days. A study

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conducted by Sohlberg and Viitanen (2014) found 5.0% growth of *Stachybotrys* sp. on light concrete supporting the possibility that *Stachybotrys* sp. can grow on concrete foundation.

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CHAPTER IV

CONCLUSIONS

When flood water enters a home, the contaminated water adsorbs into the foundation, flooring, and subflooring as well as enters the wall cavities. Once the water recedes, most homeowners remove the flooring and clean the foundation or subflooring, but questions remain on how much of the wall materials have to be removed versus what can simply be cleaned. Molds thrive in the wet humid environment left behind within a flooded wall cavity. The longer the homeowner waits before the walls and foundations are cleaned, the greater the risk of negative health effects due to exposure to molds and their associated mycotoxins. The specific aims of this research were to (1) determine which building wall materials support the highest production of mycotoxins, (2) determine under what environmental conditions the genes for mycotoxin production are being expressed, and (3) determine the concentration of mycotoxins being produced.

Mock walls were constructed from common wall materials including gypsum wallboard, fiberglass batt insulation, wood studs and oriented strand board, then these walls were flooded and exposed to the toxic mold, *Stachybotrys chartarum*. Based on DNA, the fiberglass batt insulation supported the highest concentration of fungus, followed by the gypsum paper, then the wood products. Concentrations of the fungus increased on the insulation and gypsum over time. In one sense this is surprising since fiberglass batt insulation provides no nutrients, whereas the other products all contain

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cellulose which can be consumed by the fungus. On the other hand, these same results were found in a previous study which sampled an experimental house that had been flooded (Skrobot et al. 2014). Both studies determined the greatest concentration of *S. chartarum* was on the insulation, followed by the gypsum, and this validates the residential wall set-up used in this study. It does not answer why the insulation supports such high concentrations of fungi. The prior study found that the insulation held water within the insulation after the flood water had receded, thus it may simply be that this is where the highest moisture content remains.

Of important note is that trichothecene mycotoxins were detected on all building materials at every sampling date. Trichothecene concentrations were highest on the gypsum wallboard followed by the batt insulation. So even though there was a greater concentration of fungus on the batt, there was more mycotoxin on the gypsum. In addition, DNA concentrations were very low for the wood stud and OSB, however, trichothecene concentrations were notable. Thus the mycotoxins are moving within the wall cavity and being absorbed by the other materials. The concrete paver stone also contained detectable levels of trichothecenes. Mycotoxin levels significantly increased over time. This is extremely important to homeowners, because the longer they wait to remediate their walls, the greater the exposure levels to both fungal spores and mycotoxins. Because the quality of the RNA was poor, no gene expression results could be obtained and thus there is no answer to specific aim two.

Based on this study, if a flooding event should occur within homes, it is recommended that the batt insulation and gypsum wallboard should be removed from the home. The longer these materials remain in the home, the more the mycotoxins and fungi

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will accumulate. The wood studs and oriented strand board need to be cleaned to remove the mold and tested for moisture levels. It is not known if the mycotoxins are bound to the wood stud and oriented strand board or if they could be released from the wood product back into the home. Before the wood product can be considered safe, these unknowns need to be revealed. Since there were trichothecenes detected on all building materials, one of the next research steps may be to determine what environmental factors trigger the release of the mycotoxins? Also noticing that these building materials are prone to mycotoxin accumulation, an in-depth study on the other by-products such as VOCs should be developed to determine if other mold products could cause harm to the homeowners. In summary, should a flooding event damage a home, the homeowners should remove the flooring, gypsum wallboard and batt insulation as quickly as possible, and clean the foundation, wood studs and sheathing. The longer the delay in clean-up, the greater the potential health risk to the occupants.

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APPENDIX A

METHODS

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Table A.1 Building materials inoculated with *A. fumigatus* and *S. chartarum* in preliminary study.

Sample	Sample Code	Day	Sample	Sample Code	Day
Batt Insulation	FBA15	15	**Cellulose Batt Insulation**	CBA15	15
	FBB15	15		CBB15	15
	FBC15	15		CBC15	15
	FBD15	15		CBD15	15
	FBE15	15		CBE15	15
	FBA35	35		CBA35	35
	FBB35	35		CBB35	35
	FBC35	35		CBC35	35
	FBD35	35		CBD35	35
	FBE35	35		CBE35	35
	FBA50	50		CBA50	50
	FBB50	50		CBB50	50
	FBC50	50		CBC50	50
	FBD50	50		CBD50	50
	FBE50	50		CBE50	50
	FBA65	65		CBA65	65
	FBB65	65		CBB65	65
	FBC65	65		CBC65	65
	FBD65	65		CBD65	65
	FBE65	65		CBE65	65
Plywood Sheathing	PSA15	15	**Oriented Strand Sheathing**	OSBA15	15
	PSB15	15		OSBB15	15
	PSC15	15		OSBC15	15
	PSD15	15		OSBD15	15
	PSE15	15		OSBE15	15
	PSA35	35		OSBA35	35
	PSB35	35		OSBB35	35
	PSC35	35		OSBC35	35
	PSD35	35		OSBD35	35
	PSE35	35		OSBE35	35
	PSA50	50		OSBA50	50
	PSB50	50		OSBB50	50
	PSC50	50		OSBC50	50
	PSD50	50		OSBD50	50
	PSE50	50		OSBE50	50
	PSA65	65		OSBA65	65
	PSB65	65		OSBB65	65
	PSC65	65		OSBC65	65
	PSD65	65		OSBD65	65
	PSE65	65		OSBE65	65

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Table A.2 Building materials inoculated with *S. chartarum*.

Sample	Wall	Sample Code	Day	Sample	Wall	Sample Code	Day
Oriented Strandboard	1	OSB1AA	15	**Wood Stud**	1	WS1AA	15
	1	OSB1AB	15		1	WS1AB	15
	1	OSB1AC	15		1	WS1AC	15
	1	OSB1AD	15		1	WS1AD	15
	1	OSB1AE	15		1	WS1AE	15
	2	OSB1BA	15		2	WS1BA	15
	2	OSB1BB	15		2	WS1BB	15
	2	OSB1BC	15		2	WS1BC	15
	2	OSB1BD	15		2	WS1BD	15
	2	OSB1BE	15		2	WS1BE	15
	3	OSB1CA	15		3	WS1CA	15
	3	OSB1CB	15		3	WS1CB	15
	3	OSB1CC	15		3	WS1CC	15
	3	OSB1CD	15		3	WS1CD	15
	3	OSB1CE	15		3	WS1CE	15
	1	OSB2AA	35		1	WS2AA	35
	1	OSB2AB	35		1	WS2AB	35
	1	OSB2AC	35		1	WS2AC	35
	1	OSB2AD	35		1	WS2AD	35
	1	OSB2AE	35		1	WS2AE	35
	2	OSB2BA	35		2	WS2BA	35
	2	OSB2BB	35		2	WS2BB	35
	2	OSB2BC	35		2	WS2BC	35
	2	OSB2BD	35		2	WS2BD	35
	2	OSB2BE	35		2	WS2BE	35
	3	OSB2CA	35		3	WS2CA	35
	3	OSB2CB	35		3	WS2CB	35
	3	OSB2CC	35		3	WS2CC	35
	3	OSB2CD	35		3	WS2CD	35
	3	OSB2CE	35		3	WS2CE	35
	1	OSB3AA	50		1	WS3AA	50
	1	OSB3AB	50		1	WS3AB	50
	1	OSB3AC	50		1	WS3AC	50
	1	OSB3AD	50		1	WS3AD	50
	1	OSB3AE	50		1	WS3AE	50
	2	OSB3BA	50		2	WS3BA	50
	2	OSB3BB	50		2	WS3BB	50
	2	OSB3BC	50		2	WS3BC	50
	2	OSB3BD	50		2	WS3BD	50
	2	OSB3BE	50		2	WS3BE	50
	3	OSB3CA	50		3	WS3CA	50
	3	OSB3CB	50		3	WS3CB	50
	3	OSB3CC	50		3	WS3CC	50
	3	OSB3CD	50		3	WS3CD	50
	3	OSB3CE	50		3	WS3CE	50
	1	OSB4AA	65		1	WS4AA	65
	1	OSB4AB	65		1	WS4AB	65
	1	OSB4AC	65		1	WS4AC	65
	1	OSB4AD	65		1	WS4AD	65
	1	OSB4AE	65		1	WS4AE	65
	2	OSB4BA	65		2	WS4BA	65

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Table A.2 (continued)

Sample	Wall	Sample Code	Day	Sample	Wall	Sample Code	Day
	2	OSB4BB	65	2	WS4BB		65
	2	OSB4BC	65	2	WS4BC		65
	2	OSB4BD	65	2	WS4BD		65
	2	OSB4BE	65	2	WS4BE		65
	3	OSB4CA	65	3	WS4CA		65
	3	OSB4CB	65	3	WS4CB		65
	3	OSB4CC	65	3	WS4CC		65
	3	OSB4CD	65	3	WS4CD		65
	3	OSB4CE	65	3	WS4CE		65
	1	B1AA	15	1	G1AA		15
	1	B1AB	15	1	G1AB		15
	1	B1AC	15	1	G1AC		15
	1	B1AD	15	1	G1AD		15
	1	B1AE	15	1	G1AE		15
	2	B1BA	15	2	G1BA		15
	2	B1BB	15	2	G1BB		15
	2	B1BC	15	2	G1BC		15
	2	B1BD	15	2	G1BD		15
	2	B1BE	15	2	G1BE		15
	3	B1CA	15	3	G1CA		15
	3	B1CB	15	3	G1CB		15
	3	B1CC	15	3	G1CC		15
	3	B1CD	15	3	G1CD		15
	3	B1CE	15	3	G1CE		15
	1	B2AA	35	1	G2AA		35
	1	B2AB	35	1	G2AB		35
	1	B2AC	35	1	G2AC		35
	1	B2AD	35	1	G2AD		35
	1	B2AE	35	1	G2AE		35
	2	B2BA	35	2	G2BA		35
	2	B2BB	35	2	G2BB		35
	2	B2BC	35	2	G2BC		35
	2	B2BD	35	2	G2BD		35
	2	B2BE	35	2	G2BE		35
	3	B2CA	35	3	G2CA		35
	3	B2CB	35	3	G2CB		35
	3	B2CC	35	3	G2CC		35
	3	B2CD	35	3	G2CD		35
	3	B2CE	35	3	G2CE		35
	1	B3AA	50	1	G3AA		50
	1	B3AB	50	1	G3AB		50
	1	B3AC	50	1	G3AC		50
	1	B3AD	50	1	G3AD		50
	1	B3AE	50	1	G3AE		50
	2	B3BA	50	2	G3BA		50
	2	B3BB	50	2	G3BB		50
	2	B3BC	50	2	G3BC		50
	2	B3BD	50	2	G3BD		50
	2	B3BE	50	2	G3BE		50
	3	B3CA	50	3	G3CA		50

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Table A.2 (continued)

Sample	Wall	Sample Code	Day	Sample	Wall	Sample Code	Day
	3	B3CB	50		3	G3CB	50
	3	B3CC	50		3	G3CC	50
	3	B3CD	50		3	G3CD	50
	3	B3CE	50		3	G3CE	50
	1	B4AA	65		1	G4AA	65
	1	B4AB	65		1	G4AB	65
	1	B4AC	65		1	G4AC	65
	1	B4AD	65		1	G4AD	65
	1	B4AE	65		1	G4AE	65
	2	B4BA	65		2	G4BA	65
	2	B4BB	65		2	G4BB	65
	2	B4BC	65		2	G4BC	65
	2	B4BD	65		2	G4BD	65
	2	B4BE	65		2	G4BE	65
	3	B4CA	65		3	G4CA	65
	3	B4CB	65		3	G4CB	65
	3	B4CC	65		3	G4CC	65
	3	B4CD	65		3	G4CD	65
	3	B4CE	65		3	G4CE	65

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APPENDIX B

RESULTS

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Table B.1 Real-time PCR of *A.fumigatus* DNA concentrations in nanograms per milligram of each building material on selected days.

Sample Code	Material weight (mg)	Extraction volume (mL)	Amount used µL	Real Time (ng)	Average Reps (ng)	Dilution Factor	(ng)DNA/(mg) Material	Average DNA/ Material (mg)
OSBA15	50	80	1 µL	0.00E+00		N/A	0.00E+00	
OSBB15	50	80	1 µL	0.00E+00	2.34E-02	N/A	0.00E+00	3.56E-02
OSBC15	50	80	1 µL	5.51E-03		1.00E+03	8.82E-06	
OSBD15	50	80	1 µL	3.07E-02		N/A	4.91E-02	
OSBE15	50	80	1 µL	8.07E-02		N/A	1.29E-01	
PSA15	N/A	N/A	N/A	N/A		N/A	N/A	
PSB15	N/A	N/A	N/A	N/A	0.00E+00	N/A	N/A	0.00E+00
PSC15	N/A	N/A	N/A	N/A		N/A	N/A	
PSD15	N/A	N/A	N/A	N/A		N/A	N/A	
PSE15	50	80	1 µL	0.00E+00		N/A	0.00E+00	
CBA15	100	80	4 µL	2.19E-06		N/A	4.38E-07	
CBB15	100	80	4 µL	0.00E+00	9.67E-07	N/A	0.00E+00	1.93E-07
CBC15	100	80	4 µL	9.23E-07		N/A	1.85E-07	
CBD15	100	80	4 µL	2.34E-08		N/A	4.68E-09	
CBE15	100	80	4 µL	1.70E-06		N/A	3.40E-07	
FBA15	100	80	4 µL	0.00E+00		N/A	0.00E+00	
FBB15	100	80	4 µL	0.00E+00	0.00E+00	N/A	0.00E+00	0.00E+00
FBC15	100	80	4 µL	0.00E+00		N/A	0.00E+00	
FBD15	100	80	4 µL	0.00E+00		N/A	0.00E+00	
FBE15	100	80	4 µL	0.00E+00		N/A	0.00E+00	
OSBA35	50	80	1 µL	0.00E+00		N/A	0.00E+00	
OSBB35	N/A	N/A	N/A	N/A	0.00E+00	N/A	N/A	0.00E+00
OSBC35	N/A	N/A	N/A	N/A		N/A	N/A	
OSBD35	50	80	1 µL	0.00E+00		N/A	0.00E+00	
OSBE35	50	80	1 µL	0.00E+00		N/A	0.00E+00	
PSA35	N/A	N/A	N/A	N/A		N/A	N/A	
PSB35	N/A	N/A	N/A	N/A		N/A	N/A	3.70E-01
PSC35	50	80	1 µL	8.13E-02	2.31E-01	N/A	1.30E-01	
PSD35	50	80	1 µL	6.13E-01		N/A	9.81E-01	
PSE35	50	80	1 µL	0.00E+00		N/A	0.00E+00	
CBA35	100	80	4 µL	4.84E-07		N/A	9.68E-08	
CBB35	100	80	4 µL	3.13E-07	1.00E-06	N/A	6.26E-08	2.00E-07
CBC35	100	80	4 µL	1.59E-06		N/A	3.18E-07	
CBD35	100	80	4 µL	8.84E-07		N/A	1.77E-07	
CBE35	100	80	4 µL	1.73E-06		N/A	3.46E-07	
FBA35	100	80	4 µL	0.00E+00		N/A	0.00E+00	
FBB35	100	80	4 µL	0.00E+00	4.68E-07	N/A	0.00E+00	1.56E-07
FBC35	100	80	4 µL	2.34E-06		N/A	4.68E-07	
FBD35	100	80	4 µL	0.00E+00		N/A	0.00E+00	
FBE35	100	80	4 µL	0.00E+00		N/A	0.00E+00	
OSBA50	50	80	1 µL	6.27E-02		N/A	1.00E-01	
OSBB50	50	80	1 µL	5.95E-02	4.58E-02	N/A	9.52E-02	7.33E-02
OSBC50	50	80	1 µL	0.00E+00		N/A	0.00E+00	
OSBD50	50	80	1 µL	0.00E+00		N/A	0.00E+00	
OSBE50	50	80	1 µL	1.07E-01		N/A	1.71E-01	
PSA50	50	80	1 µL	0.00E+00		N/A	0.00E+00	
PSB50	50	80	1 µL	0.00E+00	1.91E-07	N/A	0.00E+00	3.06E-07
PSC50	50	80	1µL	0.00E+00		N/A	0.00E+00	

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Table B.1 (continued)

Sample Code	Material weight (mg)	Extraction volume (mL)	Amount used µL	Real Time (ng)	Average Reps (ng)	Dilution Factor	(ng)DNA/(mg) Material	Average DNA/ Material (mg)
PSD50	50	80	1 µL	6.99E-07		N/A	1.12E-06	
PSE50	50	80	1 µL	2.58E-07		N/A	4.13E-07	
CBA50	100	80	4 µL	1.70E-06		N/A	3.40E-07	
CBB50	100	80	4 µL	0.00E+00	6.53E-07	N/A	0.00E+00	1.31E-07
CBC50	100	80	4 µL	6.06E-07		N/A	1.21E-07	
CBD50	100	80	4 µL	6.99E-07		N/A	1.40E-07	
CBE50	100	80	4 µL	2.58E-07		N/A	5.16E-08	
FBA50	100	80	4 µL	0.00E+00		N/A	0.00E+00	
FBB50	100	80	4 µL	0.00E+00	0.00E+00	N/A	0.00E+00	0.00E+00
FBC50	100	80	4 µL	0.00E+00		N/A	0.00E+00	
FBD50	100	80	4 µL	1.49E-06		N/A	2.98E-07	
FBE50	100	80	4 µL	0.00E+00		N/A	0.00E+00	
OSBA65	50	80	1 µL	9.85E-02		N/A	1.58E-01	
OSBB65	50	80	1 µL	0.00E+00	7.85E-02	N/A	0.00E+00	1.26E-01
OSBC65	N/A	N/A	N/A	N/A		N/A	N/A	
OSBD65	N/A	N/A	N/A	N/A		N/A	N/A	
OSBE65	50	80	1 µL	1.37E-01		N/A	2.19E-01	
PSA65	50	80	4 µL	0.00E+00		N/A	0.00E+00	
PSB65	50	80	4 µL	0.00E+00	0.00E+00	N/A	0.00E+00	0.00E+00
PSC65	50	80	4 µL	0.00E+00		N/A	0.00E+00	
PSD65	50	80	4 µL	0.00E+00		N/A	0.00E+00	
PSE65	50	80	4 µL	0.00E+00		N/A	0.00E+00	
CBA65	100	80	4 µL	0.00E+00		N/A	0.00E+00	
CBB65	100	80	4 µL	0.00E+00	0.00E+00	N/A	0.00E+00	0.00E+00
CBC65	100	80	4 µL	0.00E+00		N/A	0.00E+00	
CBD65	100	80	4 µL	0.00E+00		N/A	0.00E+00	
CBE65	100	80	4 µL	0.00E+00		N/A	0.00E+00	
FBA65	100	80	4 µL	0.00E+00		N/A	0.00E+00	
FBB65	100	80	4 µL	0.00E+00	0.00E+00	N/A	0.00E+00	0.00E+00
FBC65	100	80	4 µL	0.00E+00		N/A	0.00E+00	
FBD65	100	80	4 µL	0.00E+00		N/A	0.00E+00	
FBE65	100	80	4 µL	0.00E+00		N/A	0.00E+00	

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Table B.2 Real-time PCR of *S. chartarum* DNA concentrations in nanograms per milligram of each building material on selected days.

Sample Code	Material weight (mg)	Extraction volume (mg)	Amount used (mL)	Real Time (ng) µL	Average Reps(ng)	Dilution Factor	(ng)DNA/(mg) Material	Average DNA/Material
OSBA15	N/A	N/A	N/A	N/A		N/A	N/A	
OSBB15	50	80	1 µL	4.67E-06	3.75E-06	1.00E+05	7.47E-01	2.07E-01
OSBC15	50	80	1 µL	2.17E-06		1.00E+03	3.47E-03	
OSBD15	50	80	1 µL	4.46E-06		1.00E+04	7.14E-02	
OSBE15	50	80	1 µL	3.69E-06		1.00E+03	5.90E-03	
PSA15	N/A	N/A	N/A	N/A		N/A	N/A	
PSB15	N/A	N/A	N/A	N/A		N/A	N/A	9.55E-02
PSC15	50	80	1 µL	1.14E-05	1.35E-05	1.00E+03	1.82E-02	
PSD15	50	80	1 µL	1.36E-05		1.00E+03	2.18E-02	
PSE15	50	80	1 µL	1.54E-05		1.00E+04	2.46E-01	
CBA15	100	80	4 µL	0.00E+00		N/A	0.00E+00	
CBB15	100	80	4 µL	4.56E-02	4.04E-02	N/A	9.12E-03	8.08E-03
CBC15	100	80	4 µL	0.00E+00		N/A	0.00E+00	
CBD15	100	80	4 µL	1.30E-01		N/A	2.60E-02	
CBE15	100	80	4 µL	2.65E-02		N/A	5.30E-03	
FBA15	100	80	4 µL	6.20E-05		N/A	9.92E-06	
FBB15	100	80	4 µL	3.20E-04	1.43E-02	N/A	5.12E-05	2.29E-03
FBC15	100	80	4 µL	3.24E-02		N/A	5.18E-03	
FBD15	100	80	4 µL	3.32E-02		N/A	5.31E-03	
FBE15	100	80	4 µL	5.72E-03		N/A	9.15E-04	
OSBA35	N/A	N/A	N/A	N/A		N/A	N/A	
OSBB35	50	80	1 µL	0.00E+00	0.00E+00	N/A	0.00E+00	0.00E+00
OSBC35	50	80	1 µL	0.00E+00		N/A	0.00E+00	
OSBD35	50	80	1 µL	0.00E+00		N/A	0.00E+00	
OSBE35	N/A	N/A	N/A	N/A		N/A	N/A	
PSA35	50	80	1 µL	1.86E-05		1.00E+05	2.98E-01	
PSB35	N/A	N/A	N/A	N/A		N/A	N/A	7.48E-02
PSC35	50	80	1 µL	8.06E-06	2.40E-05	1.00E+02	1.29E-03	
PSD35	50	80	1 µL	0.00E+00		N/A	0.00E+00	
PSE35	50	80	1 µL	6.95E-05		N/A	1.11E-04	
CBA35	100	80	4 µL	7.30E-08		N/A	1.46E-08	
CBB35	100	80	4 µL	1.86E-02	7.24E-03	N/A	3.72E-03	1.45E-03
CBC35	100	80	4 µL	0.00E+00		N/A	0.00E+00	
CBD35	100	80	4 µL	1.76E-02		N/A	3.52E-03	
CBE35	100	80	4 µL	9.88E-08		N/A	1.98E-08	
FBA35	100	80	4 µL	5.15E-07		N/A	1.03E-07	
FBB35	100	80	4 µL	1.31E-06	1.11E-06	N/A	2.62E-07	3.14E-07
FBC35	100	80	4 µL	2.89E-06		N/A	5.78E-07	
FBD35	100	80	4 µL	1.36E-07		N/A	2.72E-08	
FBE35	100	80	4 µL	6.85E-07		N/A	1.37E-07	
OSBA50	50	80	1 µL	1.20E-05		1.00E+04	1.92E-01	
OSBB50	N/A	N/A	N/A	N/A		N/A	N/A	2.55E-01
OSBC50	50	80	1 µL	1.07E-06	1.59E-05	1.00E+04	1.71E-02	
OSBD50	50	80	1 µL	4.60E-05		1.00E+04	7.36E-01	
OSBE50	50	80	1 µL	4.61E-06		1.00E+04	7.38E-02	
PSA50	50	80	1 µL	0.00E+00		N/A	0.00E+00	
PSB50	N/A	N/A	N/A	N/A		N/A	N/A	0.00E+00
PSC50	N/A	N/A	N/A	N/A	0.00E+00	N/A	N/A	
PSD50	50	80	1 µL	0.00E+00		N/A	0.00E+00	
PSE50	50	80	1 µL	0.00E+00		N/A	0.00E+00	
CBA50	100	80	4 µL	1.02E-01		N/A	2.04E-02	
CBB50	100	80	4 µL	0.00E+00	4.10E-02	N/A	0.00E+00	8.20E-03
CBC50	100	80	4 µL	1.03E-01		N/A	2.06E-02	
CBD50	100	80	4 µL	0.00E+00		N/A	0.00E+00	
CBE50	100	80	4 µL	0.00E+00		N/A	0.00E+00	
FBA50	100	80	4 µL	7.20E-03		N/A	1.44E-03	

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Table B.2 (continued)

Sample Code	Material weight (mg)	Extraction volume (mg)	Amount used (mL)	Real Time (ng) µL	Average Reps(ng)	Dilution Factor	(ng)DNA/(mg) Material	Average DNA/Material
FBB50	100	80	4 µL	4.63E-02	1.92E-02	N/A	9.26E-03	6.36E-03
FBC50	100	80	4 µL	4.19E-02		N/A	8.38E-03	
FBD50	100	80	4 µL	5.50E-04		N/A	1.10E-04	
FBE50	100	80	4 µL	1.78E-05		N/A	3.56E-06	
OSBA65	N/A	N/A	N/A	N/A		N/A	N/A	
OSBB65	50	80	1 µL	2.83E-06	7.29E-06	1.00E+04	4.53E-02	2.18E-02
OSBC65	50	80	1 µL	6.22E-06		1.00E+03	9.95E-03	
OSBD65	50	80	1 µL	9.30E-06		1.00E+03	1.49E-02	
OSBE65	50	80	1 µL	1.08E-05		1.00E+03	1.73E-02	
PSA65	50	80	1 µL	0.00E+00		N/A	0.00E+00	
PSB65	50	80	1 µL	0.00E+00	5.39E-05	N/A	0.00E+00	1.74E-02
PSC65	50	80	1 µL	5.40E-06		1.00E+04	8.64E-02	
PSD65	50	80	1 µL	2.64E-04		N/A	4.22E-04	
PSE65	50	80	1 µL	0.00E+00		N/A	0.00E+00	
CBA65	100	80	4 µL	0.00E+00		N/A	0.00E+00	
CBB65	100	80	4 µL	2.08E-02	2.80E-02	N/A	4.16E-03	5.59E-03
CBC65	100	80	4 µL	1.14E-01		N/A	2.28E-02	
CBD65	100	80	4 µL	1.22E-03		N/A	2.44E-04	
CBE65	100	80	4 µL	3.82E-03		N/A	7.64E-04	
FBA65	100	80	4 µL	4.34E-03		N/A	8.68E-04	
FBB65	100	80	4 µL	1.99E-03	2.97E-02	N/A	3.98E-04	8.82E-03
FBC65	100	80	4 µL	1.26E-01		N/A	2.52E-02	
FBD65	100	80	4 µL	5.51E-03		N/A	1.10E-03	
FBE65	100	80	4 µL	1.09E-02		N/A	2.18E-03	

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Table B.3 Experion results for RNA quality for each building material inoculated with *A. fumigatus*.

Sample Code	Green Results (EXCELLENT) (XXXX)	Yellow Results (MODERATE) (XXX)	Red Results (POOR) (XX)	Low Concentrations (X)
OSBA15			XX	
OSBB15			XX	
OSBC15			XX	
OSBD15		XXX		
OSBE15		XXX		
PSA15				X
PSB15			XX	
PSC15				X
PSD15			XX	
PSE15			XX	
CBA15		XXX		
CBB15	XXXX			
CBC15	XXXX			
CBD15				X
CBE15				X
FBA15				X
FBB15	XXXX			
FBC15				X
FBD15				X
FBE15			XX	
OSBA35		XXX		
OSBB35		XXX		
OSBC35		XXX		
OSBD35	XXXX			
OSBE35		XXX		
PSA35			XX	
PSB35			XX	
PSC35		XXX		
PSD35		XXX		
PSE35	XXXX			
CBA35			XX	
CBB35			XX	
CBC35				X
CBD35				X
CBE35				X
FBA35			XX	
FBB35			XX	
FBC35				X
FBD35				X
FBE35				X
OSBA50	XXXX			
OSBB50		XXX		

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Table B.3 (continued)

Sample Code	Green Results (EXCELLENT) (XXXX)	Yellow Results (MODERATE) (XXX)	Red Results (POOR) (XX)	Low Concentrations (X)
OSBC50		XXX		
OSBD50		XXX		
OSBE50			XX	
PSA50		XXX		
PSB50			XX	
PSC50		XXX		
PSD50	XXXX			
PSE50			XX	
CBA50				X
CBB50				X
CBC50	XXXX			
CBD50		XXX		
CBE50		XXX		
FBA50			XX	
FBB50				X
FBC50			XX	
FBD50				X
FBE50				X
OSBA65		XXX		
OSBB65		XXX		
OSBC65		XXX		
OSBD65		XXX		
OSBE65				X
PSA65		XXX		
PSB65			XX	
PSC65			XX	
PSD65		XXX		
PSE65			XX	
CBA65			XX	
CBB65			XX	
CBC65			XX	
CBD65			XX	
CBE65			XX	
FBA65				X
FBB65			XX	
FBC65				X
FBD65				X
FBE65				X
Total	**8**	**22**	**27**	**23**

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Table B.4 Experion results for RNA quality for each building material of *S. chartarum*

Sample Code	Green Results (EXCELLENT) (XXXX)	Yellow Results (MODERATE) (XXX)	Red Results (POOR) (XX)	Low Concentrations (X)
OSBA15		XXX		
OSBB15			XX	
OSBC15			XX	
OSBD15		XXX		
OSBE15		XXX		
PSA15		XXX		
PSB15			XX	
PSC15	XXXX			
PSD15			XX	
PSE15			XX	
CBA15			XX	
CBB15			XX	
CBC15			XX	
CBD15		XXX		
CBE15			XX	
FBA15			XX	
FBB15				
FBC15				X
FBD15				X
FBE15				X
OSBA35			XX	
OSBB35		XXX		
OSBC35		XXX		
OSBD35		XXX		
OSBE35		XXX		
PSA35			XX	
PSB35			XX	
PSC35				X
PSD35			XX	
PSE35			XX	
CBA35	XXXX			
CBB35	XXXX			
CBC35	XXXX			
CBD35			XX	
CBE35			XX	
FBA35			XX	
FBB35			XX	
FBC35			XX	
FBD35				X
FBE35				X
OSBA50	XXXX			
OSBB50		XXX		
OSBC50		XXX		

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Table B.4 (continued)

Sample Code	Green Results (EXCELLENT) (XXXX)	Yellow Results (MODERATE) (XXX)	Red Results (POOR) (XX)	Low Concentrations (X)
OSBD50		XXX		
OSBE50		XXX		
PSA50			XX	
PSB50			XX	
PSC50			XX	
PSD50			XX	
PSE50			XX	
CBA50		XXX		
CBB50		XXX		
CBC50		XXX		
CBD50			XX	
CBE50			XX	
FBA50		XXX		
FBB50			XX	
FBC50		XXX		
FBD50		XXX		
FBE50		XXX		
OSBA65	XXXX			
OSBB65		XXX		
OSBC65		XXX		
OSBD65		XXX		
OSBE65		XXX		
PSA65			XX	
PSB65			XX	
PSC65			XX	
PSD65			XX	
PSE65			XX	
CBA65			XX	
CBB65			XX	
CBC65			XX	
CBD65			XX	
CBE65				X
FBA65			XX	
FBB65				X
FBC65				X
FBD65				X
FBE65				X
Total	**6**	**24**	**38**	**11**

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Table B.5 Results of ELISA for trichothecenes amounts extracted from different building materials.

Sample	Day	Optical Density	Detection of Trichothecene (y=mx+b)	Average (ppb)
Fiberglass Batt Insulation	35	2.129	BDL	
	35	1.733	BDL	
	35	2.225	BDL	0.00
	35	2.151	BDL	
	35	2.25	BDL	
	50	2.207	BDL	
	50	2.201	BDL	
	50	2.169	BDL	1.27
	50	1.979	BDL	
	50	1.18	6.33	
	65	1.915	BDL	
	65	1.777	BDL	
	65	1.686	BDL	0.00
	65	2.236	BDL	
	65	2.22	BDL	
Cellulose Batt Insulation	35	0.878	11.60	
	35	0.561	17.14	
	35	0.641	15.74	11.96
	35	1.418	2.18	
	35	0.79	13.14	
	50	0.341	20.98	
	50	1.171	6.49	
	50	1.024	9.05	13.32

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Table B.5 (continued)

Sample	Day	Optical Density	Detection of Trichothecene (y=mx+b)	Average (ppb)
	50	0.88	11.57	
	50	0.483	18.50	
	65	1.252	5.08	
	65	1.833	BDL	
	65	1.245	5.20	5.26
	65	1.24	5.29	
	65	0.927	10.75	
Plywood Sheathing	35	1.593	BDL	
	35	1.592	BDL	
	35	1.714	BDL	0.00
	35	1.803	BDL	
	35	1.815	BDL	
	50	1.525	0.31	
	50	1.746	BDL	
	50	1.628	BDL	0.06
	50	1.813	BDL	
	50	1.959	BDL	
	65	1.207	5.86	
	65	0.991	9.63	
	65	1.512	0.54	3.21
	65	1.612	BDL	
	65	1.541	BDL	
Oriented Strandboard	35	1.68	BDL	
	35	1.763	BDL	
	35	1.904	BDL	0.00
	35	1.757	BDL	
	35	1.682	BDL	
	50	1.584	BDL	
	50	1.591	BDL	
	50	1.803	BDL	1.19

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Table B.5 (continued)

Sample	Day	Optical Density	Detection of Trichothecene (y=mx+b)	Average (ppb)
	50	1.203	5.93	
	50	1.605	BDL	
	65	1.041	8.76	
	65	1.43	1.97	
	65	1.537	BDL	2.15
	65	1.561	BDL	
	65	1.903	BDL	

Samples where optical density readings were less than zero are listed as Below the Detection Limit (BDL) and were calculated as 0.0. The Limit of Detection (LOD) is 0.14ppb. The equation (y=mx+b) was used to determine level of trichothecenes.

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Table B.6 Real-time PCR of *S. chartarum* DNA concentrations results in nanograms per milligram of each building material on selected days.

Sample Code	Material weight (mg)	Extraction volume (mL)	Amount used µL	Real Time (ng)	Average Reps(ng)	(ng)DNA/(mg) Material	Average DNA/ Material (mg)
G1AA15	50	80	3 µL	0.00E+00		0.00E+00	
G1AB15	50	80	3 µL	0.00E+00	2.29E-05	0.00E+00	7.32E-06
G1AC15	50	80	3 µL	0.00E+00		0.00E+00	
G1AD15	50	80	3 µL	3.21E-05		1.03E-05	
G1AE15	50	80	3 µL	8.22E-05		2.63E-05	
B1AA15	50	80	3 µL	6.65E-07		2.13E-07	
B1AB15	50	80	3 µL	1.16E-02		3.71E-03	
B1AC15	50	80	3 µL	4.43E-02		1.42E-02	5.11E-03
B1AD15	50	80	3 µL	1.94E-02	7.10E-03	6.21E-03	
B1AE15	50	80	3 µL	4.48E-03		1.43E-03	
WS1AA15	100	80	3 µL	0.00E+00		0.00E+00	
WS1AB15	100	80	3 µL	0.00E+00		0.00E+00	
WS1AC15	100	80	3 µL	0.00E+00		0.00E+00	
WS1AD15	100	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
WS1AE15	100	80	3 µL	0.00E+00		0.00E+00	
OSB1AA15	100	80	3 µL	0.00E+00		0.00E+00	
OSB1AB15	100	80	3 µL	0.00E+00		0.00E+00	
OSB1AC15	100	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
OSB1AD15	100	80	3 µL	0.00E+00		0.00E+00	
OSB1AE15	100	80	3 µL	0.00E+00		0.00E+00	
G1BA15	100	80	3 µL	0.00E+00	6.08E-04	0.00E+00	8.27E-05
G1BB15	100	80	3 µL	2.55E-03		4.08E-04	
G1BC15	100	80	3 µL	0.00E+00		0.00E+00	
G1BD15	100	80	3 µL	3.45E-05		5.52E-06	
G1BE15	100	80	3 µL	4.54E-04		7.26E-05	
B1BA15	50	80	3 µL	8.76E-05		2.80E-05	
B1BB15	50	80	3 µL	0.00E+00		0.00E+00	
B1BC15	50	80	3 µL	0.00E+00	7.79E-05	0.00E+00	2.42E-05
B1BD15	50	80	3 µL	0.00E+00		0.00E+00	
B1BE15	50	80	3 µL	3.02E-04		9.66E-05	
WS1BA15	50	80	3 µL	0.00E+00		0.00E+00	
WS1BB15	50	80	3 µL	0.00E+00		0.00E+00	
WS1BC15	50	80	3 µL	0.00E+00		0.00E+00	
WS1BD15	50	80	3 µL	0.00E+00	0.00E+00	0.00E+00	
WS1BE15	50	80	3 µL	0.00E+00		0.00E+00	0.00E+00
OSB1BA15	50	80	3 µL	0.00E+00		0.00E+00	
OSB1BB15	50	80	3 µL	0.00E+00		0.00E+00	
OSB1BC15	50	80	3 µL	0.00E+00		0.00E+00	
OSB1BD15	50	80	3 µL	0.00E+00		0.00E+00	
OSB1BE15	50	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G1CA15	100	80	3 µL	7.95E-04		1.27E-04	
G1CB15	100	80	3 µL	2.45E-02		3.92E-03	
G1CC15	100	80	3 µL	0.00E+00	9.45E-03	0.00E+00	1.51E-03
G1CD15	100	80	3 µL	2.09E-02		3.34E-03	
G1CE15	100	80	3 µL	1.03E-03		1.65E-04	
B1CA15	100	80	3 µL	6.27E-02	1.00E-02	3.45E-03	2.14E-03
B1CB15	100	80	3 µL	1.98E-04		3.17E-05	
B1CC15	100	80	3 µL	1.79E-02		2.86E-03	
B1CD15	100	80	3 µL	6.85E-07		1.10E-07	
B1CE15	100	80	3 µL	2.71E-02		4.34E-03	
WS1CA15	50	80	3 µL	1.39E-03		4.45E-04	
WS1CB15	50	80	3 µL	5.58E-04	1.79E-04	7.83E-04	9.03E-04
WS1CC15	50	80	3 µL	0.00E+00		0.00E+00	
WS1CD15	50	80	3 µL	1.64E-03		5.25E-04	
WS1CE15	50	80	3 µL	8.64E-03		2.76E-03	
OSB1CA15	50	80	3 µL	0.00E+00		0.00E+00	

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Table B.6 (continued)

Sample Code	Material weight (mg)	Extraction volume (mL)	Amount used µL	Real Time (ng)	Average Reps(ng)	(ng)DNA/(mg) Material	Average DNA/ Material (mg)
OSB1CB15	50	80	3 µL	1.29E-03		4.13E-04	
OSB1CC15	50	80	3 µL	0.00E+00	2.64E-04	0.00E+00	8.45E-05
OSB1CD15	50	80	3 µL	3.06E-05		9.79E-06	
OSB1CE15	50	80	3 µL	0.00E+00		0.00E+00	
G2AA35	50	80	3 µL	6.53E-03		2.09E-03	
G2AB35	50	80	3 µL	1.58E-02	5.77E-03	5.06E-03	1.85E-03
G2AC35	50	80	3 µL	2.63E-03		8.42E-04	
G2AD35	50	80	3 µL	4.75E-06		1.52E-06	
G2AE35	50	80	3 µL	3.87E-03		1.24E-03	
B2AA35	50	80	3 µL	6.65E-07		2.13E-07	
B2AB35	50	80	3 µL	1.16E-02		3.71E-03	
B2AC35	50	80	3 µL	0.00E+00		0.00E+00	2.27E-03
B2AD35	50	80	3 µL	1.94E-02	7.10E-03	6.21E-03	
B2AE35	50	80	3 µL	4.48E-03		1.43E-03	
WS2AA35	100	80	3 µL	4.07E-03		6.51E-04	
WS2AB35	100	80	3 µL	0.00E+00		0.00E+00	
WS2AC35	100	80	3 µL	6.91E-05		1.11E-05	
WS2AD35	100	80	3 µL	1.11E-02	3.05E-03	1.78E-03	4.88E-04
WS2AE35	100	80	3 µL	2.59E-05		4.14E-06	
OSB2AA35	100	80	3 µL	0.00E+00		0.00E+00	
OSB2AB35	100	80	3 µL	9.66E-05		1.55E-05	
OSB2AC35	100	80	3 µL	4.35E-05	3.55E-05	6.96E-06	5.68E-06
OSB2AD35	100	80	3 µL	1.61E-05		2.58E-06	
OSB2AE35	100	80	3 µL	2.12E-05		3.39E-06	
G2BA35	100	80	3 µL	7.23E-03	2.46E-03	1.16E-03	3.93E-04
G2BB35	100	80	3 µL	1.41E-03		2.26E-04	
G2BC35	100	80	3 µL	9.30E-06		1.49E-06	
G2BD35	100	80	3 µL	3.22E-05		5.15E-06	
G2BE35	100	80	3 µL	3.60E-03		5.76E-04	
B2BA35	50	80	3 µL	5.25E-04		1.68E-04	
B2BB35	50	80	3 µL	4.71E-04		1.51E-04	
B2BC35	50	80	3 µL	9.55E-05	1.07E-03	3.06E-05	3.43E-04
B2BD35	50	80	3 µL	4.13E-03		1.32E-03	
B2BE35	50	80	3 µL	1.32E-04		4.22E-05	
WS2BA35	50	80	3 µL	3.63E-04		1.16E-04	
WS2BB35	50	80	3 µL	9.17E-04		2.93E-04	
WS2BC35	50	80	3 µL	1.01E-04	3.01E-04	3.23E-05	9.63E-05
WS2BD35	50	80	3 µL	5.45E-05		1.74E-05	
WS2BE35	50	80	3 µL	6.89E-05		2.20E-05	
OSB2BA35	50	80	3 µL	0.00E+00		0.00E+00	
OSB2BB35	50	80	3 µL	0.00E+00		0.00E+00	
OSB2BC35	50	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
OSB2BD35	50	80	3 µL	0.00E+00		0.00E+00	
OSB2BE35	50	80	3 µL	0.00E+00		0.00E+00	
G2CA35	100	80	3 µL	0.00E+00		0.00E+00	
G2CB35	100	80	3 µL	0.00E+00		0.00E+00	
G2CC35	100	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G2CD35	100	80	3 µL	0.00E+00		0.00E+00	
G2CE35	100	80	3 µL	0.00E+00		0.00E+00	
B2CA35	100	80	3 µL	1.13E-02		1.81E-03	4.62E-03
B2CB35	100	80	3 µL	0.00E+00		0.00E+00	
B2CC35	100	80	3 µL	0.00E+00	2.89E-02	0.00E+00	
B2CD35	100	80	3 µL	1.32E-01		2.11E-02	
B2CE35	100	80	3 µL	1.09E-03		1.74E-04	
WS2CA35	50	80	3 µL	1.34E-04		4.29E-05	
WS2CB35	50	80	3 µL	1.20E-04		1.92E-04	7.99E-05
WS2CC35	50	80	3 µL	2.89E-04	1.54E-04	9.25E-05	
WS2CD35	50	80	3 µL	1.21E-04		3.87E-05	

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Table B.6 (continued)

Sample Code	Material weight (mg)	Extraction volume (mL)	Amount used µL	Real Time (ng)	Average Reps(ng)	(ng)DNA/(mg) Material	Average DNA/ Material (mg)
WS2CE35	50	80	3 µL	1.04E-04		3.33E-05	
OSB2CA35	50	80	3 µL	0.00E+00		0.00E+00	
OSB2CB35	50	80	3 µL	0.00E+00	0.00E+00	0.00E+00	
OSB2CC35	50	80	3 µL	0.00E+00		0.00E+00	0.00E+00
OSB2CD35	50	80	3 µL	0.00E+00		0.00E+00	
OSB2CE35	50	80	3 µL	0.00E+00		0.00E+00	
G3AA50	50	80	3 µL	3.22E-02		1.03E-02	
G3AB50	50	80	3 µL	1.22E-06	6.44E-03	3.90E-07	2.06E-03
G3AC50	50	80	3 µL	0.00E+00		0.00E+00	
G3AD50	50	80	3 µL	3.70E-07		1.63E-05	
G3AE50	50	80	3 µL	5.24E-06		1.68E-06	
B3AA50	50	80	3 µL	2.17E-02		6.94E-03	
B3AB50	50	80	3 µL	1.69E-04		5.41E-05	
B3AC50	50	80	3 µL	9.40E-05	3.01E-05	1.47E-03	2.82E-05
B3AD50	50	80	3 µL	8.82E-05		4.59E-03	
B3AE50	50	80	3 µL	9.14E-04		2.92E-04	
WS3AA50	100	80	3 µL	6.83E-03		1.09E-03	
WS3AB50	100	80	3 µL	0.00E+00		0.00E+00	
WS3AC50	100	80	3 µL	2.72E-03		4.35E-04	
WS3AD50	100	80	3 µL	3.43E-03	2.60E-03	5.49E-04	4.15E-04
WS3AE50	100	80	3 µL	0.00E+00		0.00E+00	
OSB3AA50	100	80	3 µL	0.00E+00		0.00E+00	
OSB3AB50	100	80	3 µL	4.63E-05		7.41E-06	
OSB3AC50	100	80	3 µL	0.00E+00	9.26E-06	0.00E+00	1.48E-06
OSB3AD50	100	80	3 µL	0.00E+00		0.00E+00	
OSB3AE50	100	80	3 µL	0.00E+00		0.00E+00	
G3BA50	100	80	3 µL	2.24E-05	8.05E-04	3.58E-06	1.29E-04
G3BB50	100	80	3 µL	3.07E-03		4.91E-04	
G3BC50	100	80	3 µL	1.60E-04		2.56E-05	
G3BD50	100	80	3 µL	7.17E-04		1.15E-04	
G3BE50	100	80	3 µL	5.36E-05		8.58E-06	
B3BA50	50	80	3 µL	5.55E-03		1.78E-03	
B3BB50	50	80	3 µL	0.00E+00		0.00E+00	
B3BC50	50	80	3 µL	0.00E+00	8.83E-03	0.00E+00	2.83E-03
B3BD50	50	80	3 µL	3.86E-02		1.24E-02	
B3BE50	50	80	3 µL	0.00E+00		0.00E+00	
WS3BA50	50	80	3 µL	6.47E-04		2.07E-04	
WS3BB50	50	80	3 µL	2.92E-05		9.34E-06	
WS3BC50	50	80	3 µL	5.08E-05	9.87E-04	1.63E-05	
WS3BD50	50	80	3 µL	2.17E-03		6.94E-04	3.16E-04
WS3BE50	50	80	3 µL	2.04E-03		6.53E-04	
OSB3BA50	50	80	3 µL	0.00E+00		0.00E+00	
OSB3BB50	50	80	3 µL	2.92E-05		9.34E-06	
OSB3BC50	50	80	3 µL	6.73E-06	0.00E+00	0.00E+00	2.41E-06
OSB3BD50	50	80	3 µL	8.46E-06		2.71E-06	
OSB3BE50	50	80	3 µL	0.00E+00		0.00E+00	
G3CA50	100	80	3 µL	1.67E-06		2.67E-07	
G3CB50	100	80	3 µL	1.27E-02		2.03E-03	
G3CC50	100	80	3 µL	4.28E-05	2.55E-03	6.85E-06	4.08E-04
G3CD50	100	80	3 µL	0.00E+00		0.00E+00	
G3CE50	100	80	3 µL	1.35E-06		2.16E-07	
B3CA50	100	80	3 µL	1.64E-02	2.62E-03	4.61E-03	
B3CB50	100	80	3 µL	3.24E-02		5.18E-03	
B3CC50	100	80	3 µL	2.10E-02		2.88E-02	3.36E-03
B3CD50	100	80	3 µL	5.69E-02		9.10E-03	
B3CE50	100	80	3 µL	1.75E-02		2.80E-03	
WS3CA50	50	80	3 µL	3.45E-04		1.10E-04	

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Table B.6 (continued)

Sample Code	Material weight (mg)	Extraction volume (mL)	Amount used µL	Real Time (ng)	Average Reps(ng)	(ng)DNA/(mg) Material	Average DNA/ Material (mg)
WS3CB50	50	80	3 µL	6.99E-06	2.24E-06	2.93E-04	
WS3CC50	50	80	3 µL	8.42E-04		9.17E-04	2.69E-04
WS3CD50	50	80	3 µL	2.39E-03		7.65E-04	
WS3CE50	50	80	3 µL	1.00E-03		3.20E-04	
OSB3CA50	50	80	3 µL	0.00E+00		0.00E+00	
OSB3CB50	50	80	3 µL	0.00E+00	0.00E+00	1.54E-06	
OSB3CC50	50	80	3 µL	2.13E-05		0.00E+00	0.00E+00
OSB3CD50	50	80	3 µL	0.00E+00		0.00E+00	
OSB3CE50	50	80	3 µL	2.40E-05		7.68E-06	
G4AA65	50	80	3 µL	7.77E-04		2.49E-04	
G4AB65	50	80	3 µL	1.20E-02	9.74E-03	3.84E-03	3.12E-03
G4AC65	50	80	3 µL	1.16E-02		3.71E-03	
G4AD65	50	80	3 µL	2.39E-02		7.65E-03	
G4AE65	50	80	3 µL	4.14E-04		1.32E-04	
B4AA65	50	80	3 µL	0.00E+00		0.00E+00	
B4AB65	50	80	3 µL	0.00E+00		0.00E+00	
B4AC65	50	80	3 µL	6.58E-02	2.11E-02	1.39E-02	
B4AD65	50	80	3 µL	1.52E-01		4.36E-02	4.86E-02
B4AE65	50	80	3 µL	0.00E+00		0.00E+00	
WS4AA65	100	80	3 µL	6.83E-03		1.09E-03	
WS4AB65	100	80	3 µL	0.00E+00		0.00E+00	
WS4AC65	100	80	3 µL	2.72E-03		4.35E-04	
WS4AD65	100	80	3 µL	3.43E-03	2.60E-03	5.49E-04	4.15E-04
WS4AE65	100	80	3 µL	0.00E+00		0.00E+00	
OSB4AA65	100	80	3 µL	9.30E-05		1.49E-05	
OSB4AB65	100	80	3 µL	4.63E-05		7.41E-06	
OSB4AC65	100	80	3 µL	3.88E-05	1.26E-04	6.21E-06	2.02E-05
OSB4AD65	100	80	3 µL	3.85E-04		6.16E-05	
OSB4AE65	100	80	3 µL	6.93E-05		1.11E-05	
G4BA65	100	80	3 µL	1.46E-06	1.23E-05	2.34E-07	1.97E-06
G4BB65	100	80	3 µL	0.00E+00		0.00E+00	
G4BC65	100	80	3 µL	5.18E-05		8.29E-06	
G4BD65	100	80	3 µL	4.56E-06		7.30E-07	
G4BE65	100	80	3 µL	3.61E-06		5.78E-07	
B4BA65	50	80	3 µL	4.77E-02		1.53E-02	
B4BB65	50	80	3 µL	2.19E-02		7.01E-03	
B4BC65	50	80	3 µL	3.67E-02	2.88E-02	1.17E-02	9.22E-03
B4BD65	50	80	3 µL	2.22E-05		7.10E-06	
B4BE65	50	80	3 µL	3.77E-02		1.21E-02	
WS4BA65	50	80	3 µL	0.00E+00		0.00E+00	
WS4BB65	50	80	3 µL	8.08E-04		2.59E-04	
WS4BC65	50	80	3 µL	1.05E-02	2.29E-03	3.36E-03	
WS4BD65	50	80	3 µL	0.00E+00		0.00E+00	7.34E-04
WS4BE65	50	80	3 µL	1.55E-04		4.96E-05	
OSB4BA65	50	80	3 µL	0.00E+00		0.00E+00	
OSB4BB65	50	80	3 µL	0.00E+00		0.00E+00	
OSB4BC65	50	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
OSB4BD65	50	80	3 µL	0.00E+00		0.00E+00	
OSB4BE65	50	80	3 µL	0.00E+00		0.00E+00	
G4CA65	100	80	3 µL	0.00E+00		0.00E+00	
G4CB65	100	80	3 µL	4.48E-03		7.17E-04	
G4CC65	100	80	3 µL	2.43E-02	1.48E-02	4.86E-03	2.56E-03
G4CD65	100	80	3 µL	2.37E-06		3.79E-07	
G4CE65	100	80	3 µL	4.51E-02		7.22E-03	
B4CA65	100	80	3 µL	1.95E-01		3.12E-02	8.46E-03
B4CB65	100	80	3 µL	5.24E-05		8.38E-06	
B4CC65	100	80	3 µL	2.41E-02	5.29E-02	3.86E-03	

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Table B.6 (continued)

Sample Code	Material weight (mg)	Extraction volume (mL)	Amount used µL	Real Time (ng)	Average Reps(ng)	(ng)DNA/(mg) Material	Average DNA/ Material (mg)
B4CD65	100	80	3 µL	2.66E-05		4.26E-06	
B4CE65	100	80	3 µL	4.51E-02		7 .22E-03	
WS4CA65	50	80	3 µL	0.00E+00		0.00E+00	
WS4CB65	50	80	3 µL	0.00E+00		0.00E+00	0.00E+00
WS4CC65	50	80	3 µL	0.00E+00	0.00E+00	0.00E+00	
WS4CD65	50	80	3 µL	0.00E+00		0.00E+00	
WS4CE65	50	80	3 µL	0.00E+00		0.00E+00	
OSB4CA65	50	80	3 µL	0.00E+00		0.00E+00	
OSB4CB65	50	80	3 µL	0.00E+00	0.00E+00	0.00E+00	
OSB4CC65	50	80	3 µL	0.00E+00		0.00E+00	0.00E+00
OSB4CD65	50	80	3 µL	0.00E+00		0.00E+00	
OSB4CE65	50	80	3 µL	0.00E+00		0.00E+00	

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Table B.7 Experion results of RNA quality of building materials.

Sample Code	Green Results (EXCELLENT) (XXXX)	Yellow Results (MODERATE) (XXX)	Red Results (POOR) (XX)	Low Concentrations (X)
G1AA15			XX	
G1AB15			XX	
G1AC15			XX	
G1AD15			XX	
G1AE15			XX	
B1AA15				
B1AB15				
B1AC15	XXXX			
B1AD15				
B1AE15				
WS1AA15			XX	
WS1AB15				X
WS1AC15			XX	
WS1AD15			XX	
WS1AE15			XX	
OSB1AA15			XX	
OSB1AB15	XXXX			
OSB1AC15			XX	
OSB1AD15			XX	
OSB1AE15			XX	
G1BA15			XX	
G1BB15			XX	
G1BC15			XX	
G1BD15			XX	
G1BE15				X
B1BA15		XXX		
B1BB15			XX	
B1BC15			XX	
B1BD15			XX	
B1BE15				X
WS1BA15			XX	
WS1BB15			XX	
WS1BC15			XX	
WS1BD15			XX	
WS1BE15				X
OSB1BA15			XX	
OSB1BB15			XX	
OSB1BC15			XX	
OSB1BD15			XX	
OSB1BE15				X
G1CA15			XX	
G1CB15			XX	
G1CC15			XX	
G1CD15			XX	
G1CE15			XX	

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Table B.7 (continued)

Sample Code	Green Results (EXCELLENT) (XXXX)	Yellow Results (MODERATE) (XXX)	Red Results (POOR) (XX)	Low Concentrations (X)
B1CA15			XX	
B1CB15				X
B1CC15			XX	
B1CD15			XX	
B1CE15				X
WS1CA15			XX	
WS1CB15			XX	
WS1CC15			XX	
WS1CD15			XX	
WS1CE15			XX	
OSB1CA15		XXX		
OSB1CB15			XX	
OSB1CC15			XX	
OSB1CD15				X
OSB1CE15				X
G2AA35			XX	
G2AB35			XX	
G2AC35			XX	
G2AD35			XX	
G2AE35				X
B2AA35			XX	
B2AB35		XXX		
B2AC35			XX	
B2AD35			XX	
B2AE35			XX	
WS2AA35	XXXX			
WS2AB35			XX	
WS2AC35			XX	
WS2AD35			XX	
WS2AE35			XX	
OSB2AA35		XXX		
OSB2AB35			XX	
OSB2AC35				X
OSB2AD35			XX	
OSB2AE35			XX	
G2BA35		XXX		
G2BB35			XX	
G2BC35				X
G2BD35			XX	
G2BE35			XX	
B2BA35			XX	
B2BB35			XX	
B2BC35			XX	
B2BD35			XX	
B2BE35			XX	
WS2BA35		XXX		
WS2BB35			XX	
WS2BC35				X
WS2BD35			XX	
WS2BE35			XX	
G2CB35			XX	
G2CB35		XXX		
G2CC35			XX	

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Table B.7 (continued)

Sample Code	Green Results (EXCELLENT) (XXXX)	Yellow Results (MODERATE) (XXX)	Red Results (POOR) (XX)	Low Concentrations (X)
G2CD35			XX	
G2CE35				X
B2CA35			XX	
B2CB35				X
B2CC35			XX	
B2CD35			XX	
B2CE35				X
WS2CA35		XXX		
WS2CB35			XX	
WS2CC35			XX	
WS2CD35			XX	
WS2CE35				X
OSB2CA35		XXX		
OSB2CB35			XX	
OSB2CC35			XX	
OSB2CD35			XX	
OSB2CE35			XX	
G3AA50		XXX		
G3AB50			XX	
G3AC50				X
G3AD50			XX	
G3AE50			XX	
B3AA50			XX	
B3AB50			XX	
B3AC50			XX	
B3AD50				X
B3AE50			XX	
WS3AA50			XX	
WS3AB50		XXX		
WS3AC50			XX	
WS3AD50			XX	
WS3AE50				X
OSB3AA50			XX	
OSB3AB50			XX	
OSB3AC50			XX	
OSB3AD50			XX	
OSB3AE50			XX	
G3BA50		XXX		
G3BB50			XX	
G3BC50				X
G3BD50			XX	
G3BE50			XX	
B3BA50			XX	
B3BB50			XX	
B3BC50			XX	
B3BD50			XX	
B3BE50			XX	
WS3BA50		XXX		
WS3BB50	XXXX			
WS3BC50			XX	
WS3BD50			XX	
WS3BE50			XX	
OSB3BA50			XX	
OSB3BB50				X
OSB3BC50		XXX		
OSB3BD50			XX	

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Table B.7 (continued)

Sample Code	Green Results (EXCELLENT) (XXXX)	Yellow Results (MODERATE) (XXX)	Red Results (POOR) (XX)	Low Concentrations (X)
OSB3BE50			XX	
G3CA50			XX	
G3CB50				X
G3CC50			XX	
G3CD50			XX	
G3CE50			XX	
B3CA50				X
B3CB50			XX	
B3CC50			XX	
B3CD50			XX	
B3CE50			XX	
WS3CA50			XX	
WS3CB50		XXX		
WS3CC50			XX	
WS3CD50			XX	
WS3CE50				X
OSB3CA50	XXXX			
OSB3CB50			XX	
OSB3CC50			XX	
OSB3CD50			XX	
OSB3CE50			XX	
G4AA65		XXX		
G4AB65			XX	
G4AC65				X
G4AD65			XX	
G4AE65			XX	
B4AA65			XX	
B4AB65			XX	
B4AC65			XX	
B4AD65			XX	
B4AE65			XX	
WS4AA65			XX	
WS4AB65			XX	
WS4AC65				X
WS4AD65			XX	
WS4AE65		XXX		
OSB4AA65			XX	
OSB4AB65			XX	
OSB4AC65			XX	
OSB4AD65			XX	
OSB4AE65			XX	
G4BA65			XX	
G4BB65			XX	
G4BC65			XX	
G4BD65				X
G4BE65			XX	
B4BA65				X
B4BB65			XX	
B4BC65			XX	
B4BD65			XX	
B4BE65			XX	
WS4BA65		XXX		
WS4BB65			XX	
WS4BC65			XX	
WS4BD65				X
WS4BE65			XX	

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Table B.7 (continued)

Sample Code	Green Results (EXCELLENT) (XXXX)	Yellow Results (MODERATE) (XXX)	Red Results (POOR) (XX)	Low Concentrations (X)
OSB4BA65		XXX		
OSB4BB65			XX	
OSB4BC65			XX	
OSB4BD65			XX	
OSB4BE65				X
G4CA65			XX	
G4CB65			XX	
G4CC65			XX	
G4CD65			XX	
G4CE65			XX	
B4CA65			XX	
B4CB65			XX	
B4CC65				X
B4CD65			XX	
B4CE65			XX	
WS4CA65			XX	
WS4CB65			XX	
WS4CC65			XX	
WS4CD65				X
WS4CE65		XXX		
OSB4CA65			XX	
OSB4CB65			XX	
OSB4CC65				X
OSB4CD65			XX	
OSB4CE65			XX	

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Table B.8 Results of ELISA for trichothecenes on different building materials.

Sample Code	Wall	Optical Density (OD)	Detection of Trichothecenes (y= mx+b)	Average (ppb)
B1AA15	1	1.058	0.82	
B1AB15	1	0.637	7.91	
B1AC15	1	0.922	3.11	5.13
B1AD15	1	0.455	10.98	
B1AE15	1	0.938	2.84	
B1BA15	2	1.219	BDL	
B1BB15	2	1.167	BDL	
B1BC15	2	1.22	BDL	1.47
B1BD15	2	0.894	3.58	
B1BE15	2	0.884	3.75	
B1CA15	3	0.811	4.98	
B1CB15	3	1.37	BDL	
B1CC15	3	1.213	BDL	1.92
B1CD15	3	0.833	4.61	
B1CE15	3	1,165	BDL	
G1AA15	1	0.374	12.34	
G1AB15	1	0.137	16.33	
G1AC15	1	0.712	6.65	8.88
G1AD15	1	0.759	5.86	
G1AE15	1	0.875	3.22	
G1BA15	2	0.942	2.78	
G1BB15	2	0.363	12.52	6.73
G1BC15	2	0.639	7.88	
G1BD15	2	0.584	8.80	
G1BE15	2	1.008	1.67	
G1CA15	3	1.08	0.45	
G1CB15	3	0.636	7.93	
G1CC15	3	0.94	2.81	5.85
G1CD15	3	0.645	7.78	
G1CE15	3	0.496	10.29	
WS1AA15	1	0.916	3.22	
WS1AB15	1	0.821	4.81	
WS1AC15	1	0.924	3.08	
WS1AD15	1	0.761	5.82	4.40
WS1AE15	1	0.807	5.05	
WS1BA15	2	0.978	2.17	
WS1BB15	2	0.894	3.59	
WS1BC15	2	1.036	1.20	2.61
WS1BD15	2	0.901	3.47	
WS1BE15	2	0.951	2.63	
WS1CA15	3	1.18	BDL	
WS1CB15	3	1.419	BDL	
WS1CC15	3	0.956	2.54	1.09

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Table B.8 (continued)

Sample Code	Wall	Optical Density (OD)	Detection of Trichothecenes (y= mx+b)	Average (ppb)
WS1CD15	3	0.957	2.53	
WS1CE15	3	1.085	0.37	
OSB1AA15	1	0.881	3.80	
OSB1AB15	1	1.014	1.57	
OSB1AC15	1	0.964	2.41	
OSB1AD15	1	0.903	3.43	2.69
OSB1AE15	1	0.975	2.22	
OSB1BA15	2	1.011	1.16	
OSB1BB15	2	0.957	2.53	
OSB1BC15	2	0.983	2.09	2.47
OSB1BD15	2	0.903	3.43	
OSB1BE15	2	0.948	2.68	
OSB1CA15	3	1.138	BDL	
OSB1CB15	3	1.173	BDL	
OSB1CC15	3	1.12	BDL	1.80
OSB1CD15	3	0.632	8.00	
OSB1CE15	3	1.047	1.01	
B2AA35	1	2.712	BDL	
B2AB35	1	1.334	BDL	
B2AC35	1	1.468	BDL	0.00
B2AD35	1	1.364	BDL	
B2AE35	1	1.338	BDL	
B2BA35	2	1.08	0.79	
B2BB35	2	1.097	0.39	
B2BC35	2	1.107	0.16	0.60
B2BD35	2	1.044	1.64	
B2BE35	2	1.138	BDL	
B2CA35	3	0.99	2.90	
B2CB35	3	0.861	5.93	
B2CC35	3	1.217	BDL	3.45
B2CD35	3	0.997	2.74	
B2CE35	3	0.876	5.66	
G2AA35	1	0.93	2.98	
G2AB35	1	0.215	15.02	
G2AC35	1	0.898	3.52	5.68
G2AD35	1	0.963	2.42	
G2AE35	1	0.843	4.44	
G2BA35	2	1.105	BDL	
G2BB35	2	0.952	2.61	
G2BC35	2	0.967	2.36	2.62
G2BD35	2	0.768	5.71	
G2BE35	2	0.965	2.40	
G2CA35	3	0.87	5.72	
G2CB35	3	0.87	5.72	

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Table B.8 (continued)

Sample Code	Wall	Optical Density (OD)	Detection of Trichothecenes (y= mx+b)	Average (ppb)
G2CC35	3	1.013	2.21	5.32
G2CD35	3	0.831	6.64	
G2CE35	3	0.844	6.33	
WS2AA35	1	1.13	BDL	
WS2AB35	1	1.065	0.71	
WS2AC35	1	0.948	2.68	2.21
WS2AD35	1	1.113	BDL	
WS2AE35	1	0.652	7.66	
WS2BA35	2	0.832	6.61	
WS2BB35	2	0.828	6.71	
WS2BC35	2	0.788	7.65	6.23
WS2BD35	2	0.921	4.52	
WS2BE35	2	0.873	5.65	
WS2CA35	3	1.567	BDL	
WS2CB35	3	0.714	9.38	
WS2CC35	3	1.309	BDL	
WS2CD35	3	0.762	8.26	4.96
WS2CE35	3	0.809	7.15	
OSB2AA35	1	0.623	8.15	
OSB2AB35	1	1.114	BDL	
OSB2AC35	1	1.254	BDL	2.15
OSB2AD35	1	0.954	2.58	
OSB2AE35	1	1.197	BDL	
OSB2BA35	2	0.935	4.19	
OSB2BB35	2	0.893	5.18	
OSB2BC35	2	0.935	4.19	6.13
OSB2BD35	2	0.683	10.11	
OSB2BE35	2	0.816	6.99	
OSB2CA35	3	1.029	1.99	
OSB2CB35	3	0.685	10.06	
OSB2CC35	3	1.102	0.27	5.00
OSB2CD35	3	0.721	9.22	
OSB2CE35	3	0.967	3.44	
B3AA50	1	0.298	19.15	
B3AB50	1	0.222	20.93	
B3AC50	1	0.178	21.96	19.51
B3AD50	1	0.525	13.82	
B3AE50	1	0.189	21.71	
B3BA50	2	0.911	4.76	
B3BB50	2	1.207	BDL	
B3BC50	2	0.867	5.79	5.41
B3BD50	2	0.584	12.43	
B3BE50	2	0.941	4.05	

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Table B.8 (continued)

Sample Code	Wall	Optical Density (OD)	Detection of Trichothecene (y= mx+b)	Average (ppb)
B3CA50	3	0.339	18.19	
B3CB50	3	0.827	6.73	
B3CC50	3	0.514	14.08	14.50
B3CD50	3	0.583	12.46	
B3CE50	3	0.218	21.02	
G3AA50	1	0.243	20.44	
G3AB50	1	0.893	5.18	
G3AC50	1	1.701	BDL	13.16
G3AD50	1	0.203	21.38	
G3AE50	1	0.313	18.80	
G3BA50	2	0.489	14.66	
G3BB50	2	0.82	6.89	
G3BC50	2	0.744	8.68	10.32
G3BD50	2	0.688	9.99	
G3BE50	2	0.629	11.38	
G3CA50	3	0.335	18.28	
G3CB50	3	0.24	20.51	
G3CC50	3	0.667	10.49	17.25
G3CD50	3	0.456	15.44	
G3CE50	3	0.197	21.52	
WS3AA50	1	0.58	12.53	
WS3AB50	1	0.417	16.35	
WS3AC50	1	0.485	14.76	15.52
WS3AD50	1	0.381	17.20	
WS3AE50	1	0.4	16.75	
WS3BA50	2	0.918	4.59	
WS3BB50	2	0.761	8.28	
WS3BC50	2	0.511	14.15	7.81
WS3BD50	2	0.707	9.55	
WS3BE50	2	1.007	2.50	
WS3CA50	3	1.043	1.66	
WS3CB50	3	1.208	BDL	
WS3CC50	3	0.85	6.19	9.20
WS3CD50	3	0.774	7.97	
WS3CE50	3	0.784	7.74	
OSB3AA50	1	0.732	8.96	
OSB3AB50	1	0.718	9.29	
OSB3AC50	1	0.737	8.84	8.89
OSB3AD50	1	0.758	8.35	
OSB3AE50	1	0.608	8.98	
OSB3BA50	2	0.959	3.63	
OSB3BB50	2	1.016	2.29	
OSB3BC50	2	0.58	12.53	7.57
OSB3BD50	2	0.564	12.90	
OSB3BE50	2	0.837	6.50	
OSB3CA50	3	0.961	3.44	
OSB3CB50	3	0.604	11.96	
OSB3CC50	3	0.607	11.89	8.21
OSB3CD50	3	0.64	8.14	

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Table B.8 (continued)

Sample Code	Wall	Optical Density (OD)	Detection of Trichothecene (y= mx+b)	Average (ppb)
OSB3CE50	3	0.736	5.60	
B4AA65	1	0.419	13.99	
B4AB65	1	1.003	BDL	
B4AC65	1	0.291	17.37	9.45
B4AD65	1	0.347	15.89	
B4AE65	1	1.669	BDL	
B4BA65	2	0.226	19.09	
B4BB65	2	0.129	21.66	
B4BC65	2	0.343	16.00	18.70
B4BD65	2	0.204	19.67	
B4BE65	2	0.302	17.08	
B4CA65	3	0.413	14.14	
B4CB65	3	0.944	0.09	
B4CC65	3	0.448	13.22	12.41
B4CD65	3	0.219	19.28	
B4CE65	3	0.368	15.33	
G4AA65	1	0.39	14.75	
G4AB65	1	0.654	7.77	
G4AC65	1	0.386	14.86	14.29
G4AD65	1	0.278	17.71	
G4AE65	1	0.33	16.34	
G4BA65	2	0.076	23.06	
G4BB65	2	0.074	23.11	
G4BC65	2	0.091	22.66	22.53
G4BD65	2	0.14	21.37	
G4BE65	2	0.099	22.45	
G4CA65	3	0.141	21.34	
G4CB65	3	0.082	22.90	
G4CC65	3	0.146	21.21	21.64
G4CD65	3	0.07	23.22	
G4CE65	3	0.0209	19.54	
WS4AA65	1	0.152	21.05	
WS4AB65	1	0.182	20.25	
WS4AC65	1	0.135	21.50	20.97
WS4AD65	1	0.128	21.68	
WS4AE65	1	0.177	20.39	
WS4BA65	2	0.676	7.19	
WS4BB65	2	0.306	16.97	
WS4BC65	2	0.47	12.63	13.13
WS4BD65	2	0.495	11.97	
WS4BE65	2	0.309	16.89	
WS4CA65	3	0.441	13.40	
WS4CB65	3	0.344	15.97	
WS4CC65	3	0.408	14.28	10.57
WS4CD65	3	0.6	9.20	
WS4CE65	3	0.988	BDL	
OSB4AA65	1	0.685	6.95	
OSB4AB65	1	0.595	9.33	

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Table B.8 (continued)

Sample Code	Wall	Optical Density (OD)	Detection of Trichothecene (y= mx+b)	Average (ppb)
OSB4AC65	1	0.782	4.38	20.97
OSB4AD65	1	0.909	1.02	
OSB4AE65	1	0.497	11.92	
OSB4BA65	2	0.527	11.13	
OSB4BB65	2	0.48	12.37	
OSB4BC65	2	0.537	10.86	13.03
OSB4BD65	2	0.191	20.02	
OSB4BE65	2	0.54	10.78	
OSB4CA65	3	0.414	14.12	
OSB4CB65	3	0.945	BDL	
OSB4CC65	3	0.48	12.37	10.94
OSB4CD65	3	0.472	12.58	
OSB4CE65	3	0.359	15.57	

Samples where optical density readings were less than zero are listed as Below the Detection Limit (BDL) and were calculated as 0.0. The Limit of Detection (LOD) is 0.14ppb. The equation (y=mx+b) was used to determine level of trichothecenes.

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Table B.9 Trichothecene detection on concrete using an ELISA.

Sample Code	Optical Density (OD)	Detection of Trichothecene (y=mx+b)	Average (ppb)
1ADay15	0.966	BDL	
1BDay15	0.751	5.20	
1CDay15	0.638	8.19	4.46
2ADay35	0.574	9.88	
2BDay35	0.74	5.49	
2CDay35	0.652	7.82	7.73
3ADay50	0.807	3.72	
3BDay50	0.776	4.54	
3CDay50	0.892	1.47	3.24
4ADay65	0.894	1.42	
4BDay65	0.467	12.71	
4CDay65	0.738	5.54	6.56
5ADay 160	0.575	10.65	
5BDay 160	0.758	5.02	
5CDay 160	0. 0.935	0.33	3.91
5DDay 160	0.932	0.41	

Samples where optical density readings were less than zero are listed as Below the Detection Limit (BDL) and were calculated as 0.0. The Limit of Detection (LOD) is 0.14ppb. The equation (y=mx+b) was used to determine level of trichothecenes.

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