Mycotoxin Production by *Stachybotrys chartarum* on Water-Damaged Building Materials

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The major question after a flooding event is whether to remove or remediate the building materials so that potentially harmful mold growth and their by-products cannot cause serious health problems for susceptible individuals. The purpose of this study was to determine the growth of *Stachybotrys chartarum* and corresponding production of macrocyclic trichothecenes on different components of a residential wall up to 65 days after a simulated flood event. Small-scale residential walls constructed of fiberglass batt insulation, oriented strandboard, gypsum wallboard, and lumber were destructively sampled at four time points. All four building materials contained notable levels of macrocyclic trichothecenes on all collection days. The highest concentrations of macrocyclic trichothecenes were on the paper siding of the gypsum wallboard, followed by the paper siding of the batt insulation and wood lumber. There was a significant increase in trichothecene concentration over time, particularly on the gypsum. The DNA concentrations of the mold were significantly higher on the batt insulation than on the wood products, and the mold concentrations also increased over time on the batt insulation and gypsum, but not on the wood products. It was concluded that if a flooding event should occur, the insulation and gypsum should be removed from the home and the remaining materials should be remediated.

Keywords: Flooding; Stachybotrys chartarum; Mycotoxin; Residential wall materials

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INTRODUCTION

Hotter temperatures, heavier and more frequent rains, increased flooding, higher sea levels, and extreme weather—are all possible future natural disasters that can occur in the United States caused by climate change (NAS 2013). These outdoor changes will negatively impact the indoor environment by leading to increased moisture penetration, the decay of building materials, damage to building integrity, dampness, mold and metabolite production, and an overall increase in adverse indoor health conditions (NAS 2011; de Wilde and Coley 2012). Floods are the number one natural disaster in the United States. Since 2010, there have been ten major floods in the United States, costing Americans thirty-four billion dollars in cleanup costs and impacting all fifty states (NFIP 2016). These extreme weather events can dump up to two meters of standing water into a home. When floodwaters enter a home, and remain for extended periods of time, the floodwaters penetrate the wall cavities. After the floodwaters have receded, the building materials within the home act as a perfect substrate for mold development to occur.

Most building materials used within residential homes can support some growth of mold if moisture is present (Sivasubramani *et al*. 2004). Over the past twenty years,

residential construction procedures have changed, and buildings have become tightly sealed to minimize energy costs. This results in reduced ventilation rates but also creates ideal environments for mold. Mold enters a home from the outdoors through windows, vents, and heating and air conditioning systems. A noted difference between each material is its ability to maintain differing levels of moisture, thereby influencing the amount and type of mold. Wood and wood composites such as plywood and oriented strand board are composed of cellulose and provide the needed nutrients for mold growth. Gypsum wallboard and fiberglass batt insulation also support mold growth on their cardboard or paper backings. Andersen *et al.* (2017) evaluated gypsum wallboard for fungal growth and determined that *S. chartarum* spores might be within the paper/cardboard surrounding the gypsum during manufacturing. Building materials made of stone, such as concrete and brick, support lower levels of mold; however, the dust on these building materials can provide a nutrient source (Viitanen *et al*. 2010). The detection of mold within water damaged building materials was performed by Došen et al (2016), who found *S. chartarum* mycotoxins from both chemotypes, S and A, within dust using an ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC) method.

Different individuals have differing sensitivities to mold growth within water-damaged homes (Jarvis and Miller 2005). Both mycelia fragment and mold spores have the ability to become airborne into the home environment and eventually inhaled. In addition, some mold species produce mycotoxins. Mycotoxins are secondary metabolites that can be detrimental to the human immune system. The US Environmental Protection Agency has developed an Environmental Relative Moldiness Index that places 36 common mold species into two groups. Group 1 contains 26 species of molds associated with both water intrusion and serious health effects in susceptible individuals. Group 2 contains ten molds that have little to no impact on human health and are not associated with water intrusion within the home. Many of the molds within Group 1 have the capability to produce mycotoxins that can be transported to humans in or on the fungal spores and mycelial fragments (Vesper *et al*. 2007). Molds can produce over 300 mycotoxins, and different environmental factors stimulate toxin production (Gutarowska and Piotrowska 2007). Mycotoxins are produced by a limited number of fungal species, and are also limited to particular strains within species (Mostafa *et al*. 2012). 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. The most significant genera within water-damaged building materials that can cause serious health risks to humans include *Aspergillus fumigatus* and *Stachybotrys chartarum* (Andersen *et al*. 2011).

Because of its ability to produce potent mycotoxins and cause mycotoxicoses, *S. chartarum* is commonly referred to as the "toxic black mold" (Peska *et al*. 2008). *S. chartarum* can produce two types of trichothecene mycotoxins. One group is the toxic macrocyclic trichothecenes, whereas the second is the non-toxic simple trichothecenes (Pestka *et al*. 2008). Jarvis *et al*. (1998) estimated that one-third of *S. chartarum* isolates are able to produce toxic trichothecenes and that about two-thirds produce the nontoxic trichothecenes. The toxic macrocyclic trichothecenes are associated with serious health effects in people living or working in buildings infested with this fungus. Examples include verrucarins B and J, roridin E, satratoxins F, G, and H, and isosatratoxins F, G, and H. These mycotoxins can bind to a single site on eukaryotic ribosomes and inhibit protein synthesis. Rea *et al*. (2003) evaluated 100 patients exposed to toxic molds in 69 homes. *Stachybotrys* was found in 80% of those homes. Of 78 patients who were tested

for macrocyclic trichothecene exposure through urine analysis, 100% tested positive. Of this group, 50% tested in the high positive category. The authors concluded that *Stachybotrys* and its trichothecenes affected a person's immune, respiratory, and neurological systems. However, very few studies have established a direct causal relationship between mycotoxin exposure and building-related illness (Tuomi *et al*. 2000).

Macrocyclic trichothecenes have been found on the outside of the fungal mycelia and on the spores and are assumed to help protect the fungus from its environment (Frisvad *et al*. 1998; Täubel *et al*. 2011). However, spores of *S. chartarum* are produced in a slimy mass with high moisture content. These spores only become airborne when dry or attached to other particles such as dust. Because of this characteristic, sampling of *S. chartarum* spores or mycotoxins by an air filter or other air collection methods is often inadequate (Nieminen *et al*. 2002; Kuhn and Ghannoum 2003). Brasel *et al*. (2005) detected macrocyclic trichothecenes, in particular satratoxin G, in air samples in which particles larger than 0.4 µm had been filtered out, indicating that these toxins are easily respirable. *S. chartarum* can also be missed when strictly relying on culture isolation. It is a relatively slow-growing fungus, compared with some common flood-associated molds like *Aspergillus* sp., *Penicillium* sp., and *Paecilomyces sp.*, and thus can be overrun during culture. It should be noted that *S. chartarum* can still be viable and produce mycotoxins on dried materials (Dumon *et al*. 2009). Bloom *et al*. (2009) did not isolate *S. chartarum* from a single sample collected from homes flooded by Hurricane Katrina in New Orleans, yet detected *S. chartarum* in 100% of the samples when they were tested by PCR. However Li and Yang (2005) caution that identifying *S. chartarum* may be missed by identification and quantization using qPCR during early stages of growth and difficult to differentiate from other *Stachybotrys* species. Lewinska *et al.* (2016) used a detection method using designed primers that were able to determine *Stachybotrys* species on water-damaged building materials without the use of cultivation, DNA extraction, and DNA sequencing.

Even though some fungi have the ability to grow on different types of construction materials, mycotoxin production is highly dependent on environmental conditions, pH, and type of material (Hintikka and Nikulin 1998). The growth of *S. chartarum* and/or the production of the macrocyclic trichothecenes have been correlated with certain building materials, specifically, wallpaper and gypsum (Nielsen *et al*. 1998; Andersen *et al*. 2011). In addition, Aleksic *et al.* (2016) evaluated four different types of macrocyclic trichothecenes (satratoxins G and H, roridin L2 and verrucarin J) on different building materials. The authors quantified the trichothecenes using UPLC-MS/MS and concluded that the type of building material used within a home could potentially influence mycotoxin levels produced. Trichothecene concentrations have been shown to increase on these products as relative humidity increases (Nielsen *et al*. 1998). In contrast, Skrobot *et al*. (2013) determined that the highest concentration of *S. chartarum* in materials that had been removed from the wall of a recently flooded home was in the fiberglass batt insulation, followed by the gypsum. In fact, (Murtoniemi *et al.* 2003) showed that when fungal growth appeared on plasterboard, the bioactivity productions were low; however when the growth of the fungi was low, the spores induced major immune responses in a mouse.

The major question that needs to be answered after a flooding event is whether to remove or remediate the correct building materials so that potentially harmful mold growth and their by-products cannot cause serious health problems for susceptible

individuals. The purpose of the present work was to determine the growth of *S. chartarum* and the corresponding production of the macrocyclic trichothecenes on different components of a residential wall up to 65 days after a simulated flood event.

EXPERIMENTAL

Materials and Methods

Wall design and experimental set-up

All construction materials were obtained from Lowe's Companies, Inc. These materials included R-13 fiberglass batt insulation, oriented strand board, gypsum wallboard, and Southern Yellow Pine 2x4 lumber. Twelve 15.24 cm x 15.24 cm simulated residential walls were each constructed from two pieces of lumber, with gypsum wallboard and oriented strand board on the outer sides and the fiberglass batt insulation wedged in between, as shown in Fig. 1.

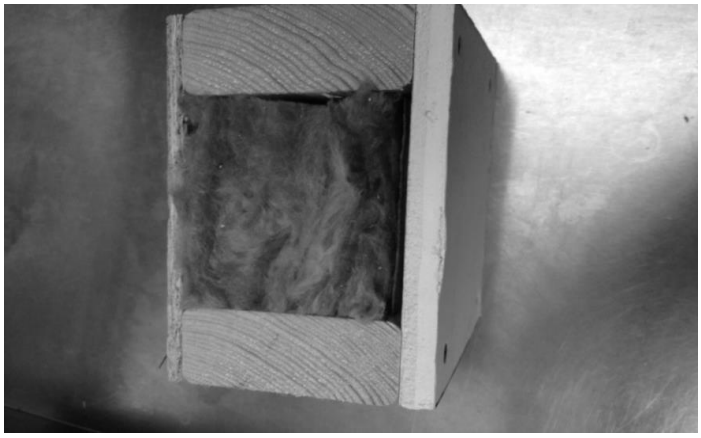

Fig. 1. Miniature model of a inside residential wall with the fiberglass batt insulation in between the 2x4 lumber

After assembly, all walls were heat treated for one hour at 100 °C before being placed into the mold chambers. The mold chamber was washed, and then 70% ethanol was used before experimentation. At the bottom of the mold chamber was a paver stone that had also been heat-treated for 1 h. The paver stone was used to simulate a concrete foundation. There were three mold chambers, each containing one wall for each collection day, plus three extras as back-ups, totaling 15 walls.

The mold chambers were flooded with 4 L of autoclaved pond water and left for three weeks at a temperature of 30 °C to simulate flooding conditions. The chambers were tightly sealed but contained a 0.2-nm filter in the lid to allow air exchange. After three weeks, approximately 2 L of the water was removed from each mold chamber. The water that remained in the mold chamber was below the top of the concrete paver stone and provided the moisture needed to maintain the humidity. Once the water was removed, the building materials were inoculated with a selected mold species.

Skrobot *et al.* (2014) showed that *S. chartarum* was dominant on different building materials and therefore selected for the current study. *S. chartarum* was isolated and cultured on potato dextrose agar. Once the plate was three-quarters covered with mycelia, 1 mL of sterile distilled water was placed on top of the mycelium and gently

scraped to dislodge the spores. The mixture was then removed from sixty-five plates and pooled to a total of 60 mL in a 100 mL flask and agitated for approximately 5 min with a stir bar. After agitation, the inoculum solution was then placed into a glass spray atomizer (Sigma, 50 mL) with compressed air. The procedure continued with the inoculation of building materials with approximately 1 mL of the suspension of *S. chartarum* (10^5 cells/mL) within the mold chamber.

At each collection day (15, 35, 50, and 65), the building wall materials were processed and assessed for both fungal growth and mycotoxin production. The wall was dismantled by first removing the batt insulation from between the woods studs. Then, the gypsum wallboard was detached from the wood studs and the paper was stripped from the gypsum itself. The wood studs were detached from the oriented strand board. All three replicate walls, on selected days, were dismantled under cold conditions (2 °C). The wood stud and OSB were scraped with a wood rasp on all sides. Finally, the gypsum front and back paper and batt insulation backing was stripped and cut with sterile scissors into small strips. For each building material, five subsample bags were collected per wall. The building wall materials used in this study were weighed (50 mg for OSB and wood stud and 100 mg for batt and gypsum), placed into 2-mL screw capped tubes, and stored at -70 °C until processing. Five subsamples of each building material were extracted for DNA and mycotoxin analysis at each collection day. This produced 60 samples that were processed for DNA quantitation and mycotoxin analysis at each of the four collection days. In addition to the building materials, the concrete paver stone was swabbed using BBL CultureSwab (Copan, Sparks, MD) and placed at -20 °C for mycotoxin analysis.

DNA isolation and qPCR analysis

Prior to the DNA extraction, all samples were freeze-dried for 1 h. Preliminary work had established that there was poor recovery of the DNA from samples that were wet. DNA extraction was conducted using the Nucleospin Plant II Kit (Machery Nagel, Duren, Germany), which included 800 µL of CTAB lysis buffer (2% cis-trimethyl ammonium boric acid, 100 mM Tris, 20 mM Na_2EDTA, 1.4 M NaCl, and 1% polyvinylpyrolidine, pH 8.0), and 20 µL of Rnase A was added. The samples were placed onto a Biospec Mini Beadbeater bead mill (Bartlesville, OK) for 3 minutes. After two cycles for 3 min at maximum speed on the beader mill, the samples were placed in a water bath at 65 °C for two hours. The DNA was extracted following the Machery Nagel instructions (Machery Nagel, Duren, Germany). The DNA samples were eluted with 80 µL of preheated PE buffer.

The DNA was amplified by PCR using an ITS1-forward primer (GTAGTCATATGCTTGTCTC) and ITS4-reverse primer (CTTCCGTCAATTCC-TTTAAG), as described by White *et al*. (1990). PCR protocols for the primers included a 4-min hot start at 98 °C, followed by 34 cycles of 95 °C for 45 s, 52 °C for 45 s, and 72 °C for 2 min, ending with a 72 °C extension for 10 min. A 2% gel electrophoresis was used to visualize the fragment amplifications. Fungal genomic DNA from *S. chartarum* mycelium was extracted as previously described in Skrobot *et al*. (2014). DNA concentrations were determined with a Nanodrop 1000 spectrophotometer (Thermo Scientific, Waltham, MA).

Quantitative polymerase chain reaction (qPCR) was used to determine the quantity of *S. chartarum* on the building wall materials. Real-time qPCR reactions were run in 96-well PCR plates with a 20-µL reaction volume in an iQ™5 Real-Time PCR Detection System (Bio-Rad, Hercules, CA). Each reaction contained 1 µL of forward and

reverse *S. chartarum* species-specific primers: forward (5'TCCCAAACCCTTATGT-GAACC-3') and reverse (5' GTTTGCCACTCAGAGAATACTGAAA-3'). These primers were designed by EPA scientists and provided by the EPA's website (ERMI 2001).
The reaction protocol included an initial denaturation at 95 °C for 2 min, followed by 45 cycles of a 15 s 95 °C denaturation, a 30 s annealing at 60 °C, and a 30 s extension at 72 °C. *S. chartarum* genomic DNA diluted in pure water was used to develop a standard curve. Threshold cycle values and concentrations were determined using Bio-Rad IQ software. After comparing the C_T and concentration values from the standard curve ranges, any values that showed poor amplifications were listed as below the detection limit. If the C_T value was above the standard curve value, the sample was diluted and re-analyzed for detection. Each value that was within the standard curve values was converted to nanograms of genomic DNA per milligram of each material.

Mycotoxin analysis

The macrocyclic trichothecenes were detected using an EnviroLogix QuantiTox kit (Portland, ME). Each material sample including the tip of the cotton swabs was placed in a 15-mL tube with 2 mL of phosphate buffered saline and mixed on an orbital shaker for ten minutes at 200 rpm. After shaking, 1 mL of supernatant from each sample was placed into a 1.5-mL tube and centrifuged for 5 min at 10,000 rpm. A 900 ppb Roridin A stock solution was prepared as directed by the kit and diluted to 18.0 ppb, 2.0 ppb, and 0.2 ppb in PBS buffer. Once all components reached room temperature, 50 µL of negative calibrator, 50 µL of Roridan A calibrator, and 50 µL of each sample extract were added to their respective designated wells. After 50 µL of enzyme conjugate was immediately added to each well, the protocol for the kit was followed. Each plate containing samples and the Roridin A standard curve was read on an Epoch Microplate Spectrophotometer (BioTek, Winooski, VT) set to 450 nm. The non-inoculated materials were also assessed to ensure there was no background interference from the materials. The EnviroLogix measures the concentration of Roridin E, which is the precursor for all of the toxic macrocyclic trichothecenes, and thus the results reflected the total macrocyclic trichothecenes rather than an individual type. The detection limit of this kit is 0.14 ppb.

Statistical analysis

The genomic DNA samples were converted to nanograms per milligram of material, and statistical differences were determined for the different building materials. An analysis of variance (ANOVA) with Tukey's test run on SAS 9.4 (SAS Institute, Cary, NC) was used to determine if the means of the different building materials were significantly different. Statistical differences were also determined for the mycotoxin data as a comparison of means among the different building materials for each collection day as well as over time.

RESULTS AND DISCUSSION

Based on the qPCR results, the fiberglass batt insulation supported the highest concentration of *S. chartarum*, followed by the gypsum wallboard paper, then the wood products (Table 1).

Table 1 shows that the concentrations of *S. chartarum* were statistically greater on the insulation than on either the wood stud or the OSB, whereas there was no significant difference between the gypsum and the other materials. These results were somewhat surprising, as the presence of *S. chartarum* is often associated with water-damaged gypsum. Nielsen *et al*. (1998) found that all isolates of *S. chartarum* that had been artificially inoculated onto different building materials grew best on gypsum wallboard, while there was no growth on chipboard, acoustic tiles, or pinewood. A similar study with *S. chartarum* obtained the best growth on gypsum wallboard and wallpaper, but there was some growth on pine paneling and paper (Nikulin *et al.* 1994). Andersen *et al*. (2011) ran principle component analysis on over 5,300 surface samples taken from water-damaged buildings in Denmark and correlated the species to the material source. These authors found a strong association between the presence of *S. chartarum* and gypsum wallboard, wallpaper, and glass fiber wallpaper. In this case, the glass fiber was not insulation.

In contrast, Skrobot *et al*. (2013) used qPCR to quantify the presence of molds from wall materials removed from a water-damaged simulation home. In this study, materials were removed three weeks after flooding and included fiberglass batt insulation, gypsum wallboard, plywood sheathing, pine wood stud, house wrap, and vinyl siding. The concentrations of *S. chartarum* were detected on all materials except the wood stud, house wrap, and vinyl siding. The highest concentration was on the fiberglass batt insulation, followed by the gypsum. Very low concentrations were detected on the plywood. One reason the batt insulation may support high concentrations of *S. chartarum* is that it also holds high levels of moisture and has a paper backing. It may be possible that once it is water damaged, the paper backing acts as a possible nutrient source for microbial growth such as mold. When packed within the wall, the interwoven fibers retain moisture. The moisture levels of the batt insulation removed from the same study three weeks after the water had drained was still at 100% (Aglan *et al*. 2014). *S. chartarum* is considered a tertiary colonizer, requiring water activity greater than 0.9 (Grant *et al*. 1989), and thus would thrive in a very moist environment.

In the current study, the concentrations of the fungus increased on the batt insulation and gypsum over time, but not on the wood products, as shown in Fig. 2. The figure shows a particularly large jump in concentration from day 50 to day 65 on the batt insulation. The statistical analysis of *S. chartarum* DNA concentrations on different collection dates averaged across all materials for each date found no statistically significant difference among the dates. This was likely due to the high variability among subsamples, which could have masked possible differences. One disadvantage of qPCR is its extreme sensitivity. This sensitivity is also an advantage when testing for *S. chartarum* from samples collected in the field. As stated previously, *S. chartarum* is often absent in culture isolations, yet is detectable by qPCR (Bloom *et al*. 2009).

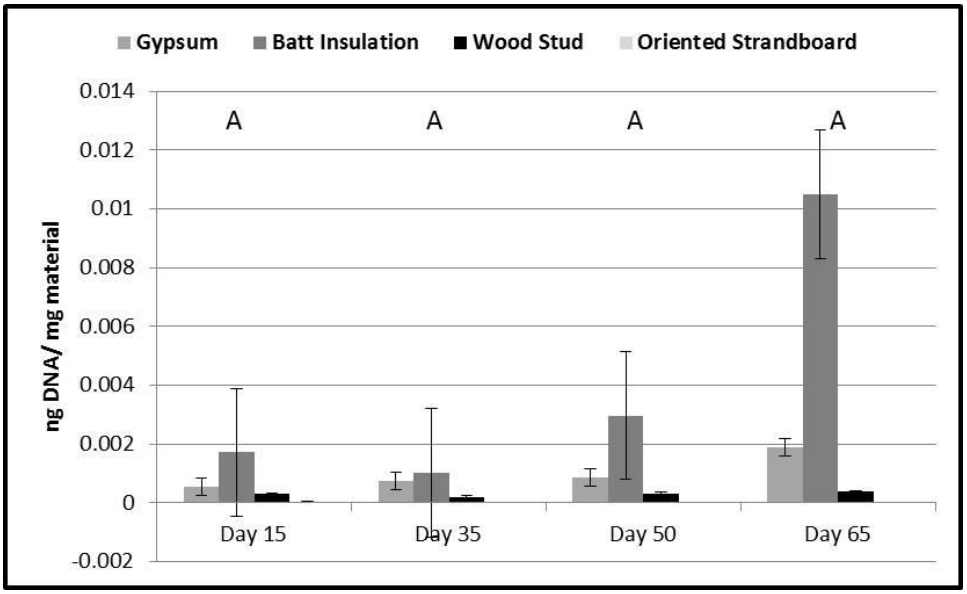

Fig. 2. *S. chartarum* DNA concentrations (ng DNA/mg material) on building wall materials for each collection date. Error bars represent the standard error of the mean. Means with the same letter are not significantly different at the p = 0.05 level when all materials were averaged at each collection date.

Trichothecenes were detected on all of the different building materials for each day tested (Fig. 2). The highest concentrations were on the gypsum wallboard paper, followed by the wood stud and batt insulation. The trichothecene concentrations were significantly greater on the gypsum compared to the oriented strandboard and concrete paver in Table 1.

Table 1. Statistical Analysis of *S. chartarum* DNA and Trichothecene Concentrations on Different Building Materials Averaged Across All Dates Collected

Building Material	Average of Trichothence Concentrations Mean =12	Tukey Grouping of Trichothecene Concentrations	Average of DNA Concentrations Mean N=12	Tukey Grouping of DNA Concentrations
Fiberglass Insulation	7.784	AB	0.00725	A
Gypsum Wallboard	11.543	A	0.00101	AB
Wood Stud	8.173	AB	0.00031	B
Oriented Strandboard	6.565	B	0.00001	B
Concrete	5.498	B		

Means with the same letter are not significantly different at the P = 0.05 level

Table 1 also shows that the batt insulation had the greatest concentration of the fungus. It is well documented that *S. chartarum* often prefers gypsum, and thus high levels of toxin on the gypsum is not surprising. Nielsen *et al*. (1998) detected the

verucarol-type of trichothecenes on gypsum wallboard inoculated with *S. chartarum*, while Nikulin *et al*. (1994) measured satratoxins G and H on wallpaper and gypsum inoculated with *S. chartarum* growing at high relative humidity. Interestingly, the same study found high levels of *S. chartarum* on pine panels at 100% relative humidity, but detected no toxins on the pine. In the current study, DNA concentrations were very low on the wood stud and OSB; however, trichothecenes were detected, particularly on the wood stud.

Several studies have measured the presence of different trichothecenes from air, dust, and interior wall samples; however, these studies did not always detect *S. chartarum* when mycotoxins were present (Tuomi *et al*. 2000; Bloom *et al*. 2009; Polizzi *et al*. 2009). It is also worth noting that in this current study, trichothecenes were present on the concrete. A study conducted by Sohlberg and Viitanen (2014) found 5.0% growth of *Stachybotrys sp*. on light concrete. The increase in mycotoxin levels over time is shown in Fig. 3.

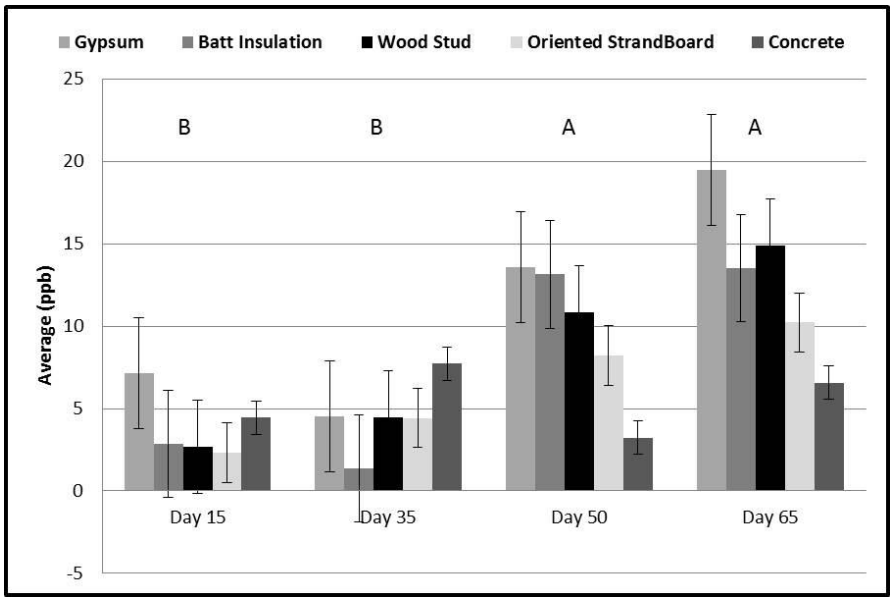

Fig. 3. *S. chartarum* DNA concentrations (ng DNA/ mg material) on building wall materials for each collection date. Error bars represent the standard error of the mean. Means with the same letter are not significantly different at the p = 0.05 level when all materials were averaged at each collection date.

Table 2. Statistical Comparison of Trichothecene Concentrations Averaged for Each Material and for Each Date

Material	Day 15	Day 35	Day 50	Day 65
Gypsum	BCD	BCD	ABC	A
Wood Stud	BCD	BCD	ABCD	AB
Batt Insulation	CD	D	ABC	ABC
Oriented Strandboard	CD	BCD	BCD	ABCD
Concrete	BCD	BCD	CD	BCD

Means with the same letter are not significantly different at the p = 0.05 level.

There was an overall significant increase at days 50 and 65 compared with days 15 and 35. This could be extremely important to homeowners because it implies that the longer the fungus stays in the walls, the more likely the mycotoxin levels will increase unless remediation is performed, as shown in Fig. 2. The comparison for each material at each time is shown in Table 2.

The table shows a significant increase in trichothecene concentrations on gypsum at day 65 compared with days 15 and 35. Trichothecene levels on the fiberglass batt insulation at day 35 were significantly lower compared with days 50 and day 65. There were no significant changes over time for the wood stud, OSB, and concrete. The trichothecene concentrations on gypsum at day 65 were significantly higher than those of all other materials at days 15 and 35, OSB at day 50, and concrete at all days tested. The overall trichothecene concentrations varied depending on material and day tested.

CONCLUSIONS

1. When a home is damaged by water, the water can be contaminated with foreign materials, such as oil, dirt, and sewage, which can be adsorbed into the foundation, flooring, and subflooring as well as enter the wall cavities. Once the water recedes, the cleanup by the homeowner involves the removal of the flooring and cleaning the foundation of the house. Gypsum wallboard is often removed after a flood because both the integrity and the appearance of the material have been negatively impacted by the floodwaters.
2. This study has shown that both the fiberglass batt insulation and gypsum wallboard supported a high concentration of the mold S. *chartarum* and also harbored toxic trichothecene mycotoxins. Concentrations of the mold and concentrations of the mycotoxin increased over time on both of these wall materials. In addition, mold concentrations were very low on the wall wood products; however, trichothecene concentrations were notable. This implies that the mycotoxins were moving within the wall cavity and being absorbed by the other materials. It is not known if the mycotoxins are bound to the wood stud and oriented strandboard or if they could be released from the wood product back into the home.
3. This study has demonstrated that the longer the homeowner waits before the walls and foundations are cleaned, the greater the risk of jeopardizing the indoor air quality. If a flooding event should occur, it is recommended that the batt insulation and gypsum wallboard be removed from the home. The longer these building materials stay within the home, the more mycotoxins and fungi can accumulate.
4. The remaining wall materials need to be remediated with a cleaning solution (Wilson *et al*. 2004). Because trichothecenes were detected on all building materials, including concrete, all remaining materials must be thoroughly cleaned. The longer the delay in cleanup is, the greater is the potential health risk to the occupants.

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