FUNGAL POPULATIONS IN AIR AND MATERIALS IN A FLOOD SIMULATION STUDY

Frederick (Trey) Skrobot III†
Graduate Student
Department of Sustainable Bioproducts
Mississippi State University
Mississippi State, MS 39762-9820
E-mail: fs54@msstate.edu

Heshmat A. Aglan
Professor and Associate Dean
College of Engineering
Tuskegee University
Tuskegee, AL 36088
E-mail: aglanh@mytu.tuskegee.edu

Shane Kitchens†
Assistant Professor
Department of Sustainable Bioproducts
Mississippi State University
Mississippi State, MS 39762-9820
E-mail: skitchens@cfr.msstate.edu

Adriane Ludwick
Professor
Department of Chemistry
Tuskegee University
Tuskegee, AL 36088
E-mail: aludwick@mytu.tuskegee.edu

Terry Amburgey†
Professor Emeritus
E-mail: terryamburgey@yahoo.com

Hamid Borazjani
Professor
E-mail: hborazjani@cfr.msstate.edu

*Susan V. Diehl**†
Professor
Department of Sustainable Bioproducts
Mississippi State University
Mississippi State, MS 39762-9820
E-mail: sdiehl@cfr.msstate.edu

(Received January 2014)

* Corresponding author
† SWST member

Wood and Fiber Science, 46(4), 2014, pp. 465-479

Abstract. Air quality was measured in a building subjected to flooding conditions analogous to that which occurred during Hurricane Katrina. This building was flooded to a depth of 0.61 m above the floor with pond water and maintained at that level for 3 wk. After the floodwater was drained, the building remained closed for an additional 3 wk. Immediately on opening, air samples were obtained and analyzed for fungal spores. Dry and wet material components of the building wall were analyzed for the presence of mold fungi by both culture and molecular techniques. Additional air samples were taken after a 30-da drying period and then after remediation of the building. The air measurements demonstrated the presence of high concentrations of indoor mold spores when the building was initially entered. *Aspergillus*/*Penicillium* were the dominate air molds. Fiberglass batt insulation supported the greatest concentration of culturable fungi, compared with other wall materials, followed by the paper facings of gypsum board and plywood sheathing. The solid wood stud, vinyl siding, and house wrap all supported low concentrations of culturable mold. After drying, the spore air contamination diminished more than 10-fold and the species of fungi on the materials drastically changed. After remediation, the spores inside the structure nearly matched those outside with respect to type and concentration.

Keywords: Mold, floods, indoor air quality, remediation.

INTRODUCTION

As climate change impacts the southeastern US, increased flooding, more storm surges, and higher sea levels are probable consequences. Worldwide, more than 115 million people are affected by floods and more than 9000 are killed annually (NAS 2013). Excess moisture, long periods of heat and humidity, and ponding of water are factors often associated with floods and provide suitable conditions for fungal (mold) growth in building materials. When flood waters enter a home and remain for an extended period of time, the water penetrates the wall cavities. As flood waters recede, the different wall materials such as the wood studs, wood sheathing, ceiling tiles, wallpaper, paint, carpet, sheet rock, and insulation can serve as a substrate for mold development. It is probable that most building materials can support the growth of mold at some level; however, differences in water-holding capacity and organic/inorganic composition will influence the amount and type of mold. A compilation of several different studies suggests that 47% of residents in US homes or housing units are exposed to increased dampness and mold (Mudarri and Fisk 2007).

A National Academy of Sciences (NAS 2004) report on dampness and mold suggested that there is enough scientific evidence to support an association among indoor dampness, mold, and certain adverse health effects of the residents. Health problems that have been associated with mold exposure can be categorized into short-term and long-term effects. Short-term effects include irritations and general symptoms such as rhinitis, sore throat, hoarseness, cough, phlegm, shortness of breath, eye irritation, eczema, tiredness, headache, nausea, difficulties in concentration, and fever (Koskinen et al 1999; Park et al 2004; Brandt et al 2006; Cabral 2010). Long-term effects include infections such as the common cold, otitis, maxillary sinusitis, bronchitis, and allergic diseases such as simple allergies, asthma, and alveolitis. The most common health effects are associated with the respiratory system. A meta-analysis of the data by Fisk et al (2007) associated exposure to dampness and mold with a 30-50% increase in respiratory and asthma-related health problems. An estimated 4.6 million cases of asthma in the US result from exposure to indoor dampness and mold, which results in an estimated $3.5 billion annual economic health cost (Mudarri and Fisk 2007).

Many different molds have been associated with water-damaged homes and negatively impact the indoor environment (Prezant et al 2008; Samson et al 2010; Flannigan and Miller 2011; Miller 2011). The US Environmental Protection Agency (EPA) has developed the Environmental Relative Moldiness Index as a way to compare levels or scales of moldiness (Vesper et al 2007a, 2007b). This index is based on DNA

analysis for the presence of 36 mold species. These species have been divided into two groups. Group 2 contains 10 species of common indoor molds that do not appear to be associated with negative health effects nor associated with water intrusion. Group 1 contains 26 species of molds that are associated with both water intrusion and significant adverse health effects. Many molds in Group 1 produce mycotoxins that can be transported to humans in or on the fungal spores and fragments. However, just because these species are present in a water-damaged home does not mean these species are producing mycotoxins. There is still insufficient information linking mycotoxin production within damp indoor spaces and adverse impact on human health. Volatile organic compounds (VOCs) are also produced by molds, often resulting in a strong odor. The extent of VOC contamination is a function of the severity of the mold contamination as well as the type of mold. Also associated with mold and adverse human health are fragments of the fungal cell wall known as glucans or β-(1-3)-D-glucans. High levels of these fragments within the dust of water-damaged homes can cause flu-like illnesses, coughs, and hoarseness (Rylander et al 1998).

A distinction needs to be made between homes damaged by interior or exterior water leaks and homes damaged by floods. 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. This water is often heavily laden with sediments, sewage, fertilizers, and pesticides and provides a breeding ground for fungi, bacteria, and other microorganisms. In the wake of Hurricanes Katrina and Rita, numerous studies evaluated flood-damaged homes for elevated mold spore levels, culturable fungi, glucans, and endotoxins (Chew et al 2006; Solomon et al 2006; Rao et al 2007; Riggs et al 2008; Adhikari et al 2009, 2010; Bloom et al 2009). In addition to mold and fungal-related products, flooded homes can also be a significant source of endotoxins, which are lipopolysaccharide cell wall components from Gram-negative bacteria. Endotoxins can also contribute to adverse respiratory ailments in exposed residents (Pirie et al 2003). Riggs et al (2008) evaluated 112 homes in New Orleans after the hurricanes and found that 64% were flooded with 19% flooded with greater than 1.8 m of water, 19% flooded with 0.9-1.8 m of water, and 26% flooded with less than 0.9 m of water. Visible mold, culturable mold, and spore numbers were all significantly greater in homes with flood levels higher than 0.9 m. Endotoxins and glucans were higher in all flooded homes, regardless of flood levels, compared with nonflooded homes. Riggs et al (2008) also found that concentrations of all of their microbial measurements were higher in homes with gypsum drywall vs plaster and with tile or concrete floors vs carpet. Culturable *Aspergillus* levels were significantly less on plaster vs gypsum drywall, whereas the levels of culturable *Penicillium* were not different. Barbeau et al (2010) also found that homes with higher levels of flood waters (>0.9 m) contained higher levels of mold. Extrapolating their data, Riggs et al (2008) estimated that 194,000 homes contained elevated levels of mold following the hurricanes of 2005 with 70,000 of these contaminated with heavy mold growth.

The extent and diversity of microbial contamination after floods are vast compared with normal moisture-related building problems such as leaks and condensation. This complexity makes it difficult to obtain a comprehensive picture of the contamination. There is no general agreement on the best methods, conditions, or interpretation of microbial sampling techniques, thus multiple sampling techniques are recommended (NAS 2004; Niemeier et al 2006; Samson et al 2010).

The purpose of this study was to examine the effect of long-term flooding and subsequent drying on the extent and type of mold in the air and on the different building materials that make up residential walls. Results from this same study on the moisture and mechanical integrity of the wall materials have been published elsewhere (Aglan et al 2013).

MATERIALS AND METHODS

Flood Unit

All construction materials were obtained commercially in proximity to Tuskegee, AL. These materials included wood (38-mm × 89-mm × 2.44-m southern yellow pine studs, 12.7-mm exterior-grade plywood panels), gypsum board, house wrap (nonwoven high-density polyethylene), fiberglass batt insulation (R-13), vinyl siding, and roofing shingles. The 3.66-m × 3.66-m × 2.44-m unit was constructed by Sippial Electric and Construction Co., Tuskegee, AL. The framing (2 × 4 untreated lumber) was placed on a concrete slab foundation. Plywood panels were placed to the outside of the frame and gypsum boards to the inside with the insulation between these panels and the house wrap between the plywood and siding. Vinyl siding was used to complete the outside, and the gypsum boards were painted with water-based paint. Vents were placed on the south and north faces of the unit. One window was placed on the north, east, and west sides. The door was on the south side.

The flood unit was in proximity to a farm pond used by cattle. Near the pond was a pump that transferred the pond water through hosing to the area surrounding the flood unit (Fig 1). The pond water surrounded and entered the flood unit (0.61 m deep above floor level) for a period of 3 wk (May 10 to May 31, 2010). Water samples were collected just prior to drainage and sent to Mississippi State University (MSU) for microbial analysis. The pond water was then drained and the unit remained untouched for another 3 wk (June 1 to June 21). Three types of sensors were embedded into the walls of the flood unit. The wiring from these sensors went through the vents on the north and south walls of the unit to a control room located in proximity to the flood unit. Combined temperature–humidity sensors (Vaisala Model EW-03334-05, Edison, NJ) were placed inside the cavity of the south wall and inside the unit. Measurements were taken every 20 min during flooding and every 2 h after opening. Moisture pins (Delmhorst KIL-MO-TROL, Towaco, NJ) were installed in one stud in the south wall at 0.46 and 0.77 m above floor level. Moisture content was recorded every 2 h during both the flooding and drying periods. Moisture in the gypsum board was measured using a handheld moisture sensor (Delmhorst) before flooding, on re-entry after draining, and throughout the drying period. The flood unit was opened on June 21, 2010. Remediation of the mold-contaminated flood unit was done by SERVPRO on 12 August 2010.

Figure 1. Flood unit built at Tuskegee University at flood stage. Unit is surrounded by 0.91 m of water from a nearby pond (in back of photograph). After 3 wk of flooding, the water was drained and the unit remained closed for an additional 3 wk prior to sampling.

Air Sampling

Following the EMSL Air-O-Cell sampling guide (EMSL 2012), Air-O-Cell cartridges (Zefron International, Ocala, FL) were used to collect fungal spores and particulates from the outside air prior to opening the flood unit and within the flood unit just after opening. Additional readings were taken 1 mo after entry (July 21, 2010) and immediately after remediation (August 13, 2010). Sample volumes ranged from 75 to 150 L. These samples were sent to EMSL Analytical, Inc. (Westmont, NJ) for analysis using phase contrast microscopy at 600 × and 300 × total magnifications. Thirty percent of the trace, with a 300 spore-stopping rule for each individual spore type, was analyzed at 600× followed by 100% of the trace at 300×. Spore trap results were reported in spores per cubic meter with

background particle density rated (EMSL Method M001). Spores were classified by morphological characteristics including size, shape, color, surface texture, ornamentation, and fruiting structures (if present), which were compared with published mycological identification keys and texts (EMSL Analytical, Inc.).

Materials and Pond Water Sampling

Wall material samples were removed from the south wall of the flood unit on initial entry following drainage of the flood water and were sent to MSU for mold analyses immediately after opening (wet samples) and approximately 7 mo after opening (dry samples) (February 1, 2011). Wet material samples included four samples of gypsum board (varying 300-460 mm wide × 800-900 mm long), fiberglass batt insulation (460 × 900 mm), wood studs (800 mm long), wood sheathing, vinyl siding, and house wrap (460 × 800 mm). Dry material samples included gypsum board and wood studs. Material sample location and exposure are given in Table 1. Samples of materials were removed from various locations, including front and back when possible, as well as above and below the water line. Small pieces (sizes are described subsequently) of these materials were either placed into 9 mL of sterile water or placed directly on culture media. Samples in water were vortexed aggressively to dislodge particulates, and 0.1 mL of water suspension was spread on potato dextrose agar amended with 0.3 g chlortetracycline and 0.12 g/L^{-1} streptomycin sulfate. This media was selected because it is known to be a better media for growing the hard to grow molds such as *Stachybotrys* (Samson et al 2010), whereas the addition of antibiotics inhibits bacteria. As colonies formed, cultures were transferred to fresh media and grouped based on visual appearance. Cultures were recultured on fresh plates until a single colony type was present per plate. Pure cultures were transferred to potato dextrose broth and grown at room temperature on a shaker until sufficient mycelia had formed. Mycelia were filtered and frozen at −20°C. Tuskegee pond water was directly spread on the same type of media, and fungal cultures that developed were isolated and processed as mentioned. Moisture content of the wall materials was also estimated by the oven-dry method.

Small pieces from above and below the water line were separated for each wet and dry material sample and frozen at −20°C. Gypsum samples were separated into front white paper, brown paper backing (approximately 5-8 mm

Table 1. Material samples exposed to flood waters that were sent to Mississippi State University for mold analysis.[a]

Material		Exposure
Gypsum board	Wet material	Above and below water line
Gypsum board		Above water line
Gypsum board	With sample #G1	Above and below water line
Batt insulation[b]		Above water line
Batt insulation[b]		Below water line
Gypsum board	Sample #7	Above and below water line
Wood stud[c]	Sample #7	Above and below water line
Plywood	Sample #7	Above and below water line
House wrap	Sample #7	Above and below water line
Vinyl siding	Sample #7	Above and below water line
	Dry material	
Gypsum board		Above water line
Gypsum board		Below water line
Wood stud		Above water line
Wood stud		Below water line

[a] Wet samples were removed from the south wall just after opening the flood unit on June 21, 2010. Dry samples were removed 7 mo later on February 1, 2011.
[b] Fiberglass insulation.
[c] Southern Yellow Pine.

square), and gypsum. Batt insulation was separated into paper fronting and insulation (approximately 5-8 mm square). Wood studs and plywood sheathing were rasped into small pieces from the front and back sides (lengths and widths varying from 2 to 15 mm). Vinyl siding and house wrap were not separated except by exposure and cut down to 5- to 8-mm square pieces. The species of molds isolated and identified on the different wall materials were not an exhaustive screening. The pieces of wall materials cultured were very small with respect to the material sample size, and we were not able to isolate and identify every fungal colony on every plate. Thus, it is likely that some of the fungal species identified on certain wall materials may also have been present on the other wall materials; they simply were not cultured from the specific pieces chosen. The purpose of this screening was to identify as many different species of molds present on the wall materials as possible.

Mold Identification

Mycelia (0.05 g) from pure cultures were extracted for DNA using the Nucleospin Plant II kit protocol for fungi (Macherey-Nagel, Düren, Germany). DNA fragments were amplified by polymerase chain reaction (PCR) using NS1F and NS4R (White et al 1990) and/or ITS1F and ITS4R (Gardes and Bruns 1993). PCR protocols for the NS 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. PCR protocols for the ITS primers included a 4-min hot start at 94°C followed by 39 cycles of 94°C for 35 s, 55°C for 55 s, and 72°C for 1 min, ending with a 72°C extension for 10 min. Fragment DNA was cleaned using the Nucleospin Extract II kit following the protocol for direct purification of PCR products (Macherey-Nagel). Fragment DNA samples were prepared for sequencing following the Beckman Coulter dye terminator cycle sequencing protocol using the sample appropriate forward or reverse primer (Beckman Coulter, Fullerton, CA). Both forward and reverse fragments were sequenced for each sample using a Beckman Coulter CEQ 8000 capillary sequencer. All sequence data were checked for quality, and the forward and reverse sequences for each sample were aligned using LaserGene MegAlign software (DNASTAR, Madison, WI). The consensus sequence for each sample was submitted to a GenBank Blast search for match identifications.

Select material samples were also extracted for DNA using the Nucleospin Plant II kit protocol for plants (Macherey-Nagel) modified using CTAB buffer. Fragments were amplified by PCR using the primers and protocols previously listed. Because these materials were environmental samples and not pure cultures, they contained multiple species DNA. Therefore, it was necessary to clone the samples before sequencing could be performed. Amplified fragments were cloned into *Escherichia coli* vectors using the TOPO TA cloning kit following kit procedures (Invitrogen, Carlsbad, CA). Twenty clones were extracted for each sample using the PureLink quick plasmid miniprep kit (Invitrogen). Clones containing inserts were verified by a 1-h *EcoR1* digest followed by visualization on a 1.5% agarose gel. Samples containing inserts were prepped for sequencing following the Beckman Coulter dye terminator cycle sequencing protocol using the T3 and T7 plasmid primers (Beckman Coulter). Both forward and reverse fragments were sequenced for each sample using a Beckman Coulter CEQ 8000 capillary sequencer. All sequence data were checked for quality and the plasmid DNA was removed from the sequence ends. The forward and reverse sequences for each sample were aligned using LaserGene MegAlign software. The consensus sequence for each sample was submitted to a GenBank Blast search for match identifications.

RESULTS AND DISCUSSION

Air Sampling Results

Air quality was assessed at three time periods. Most mold spores found in the air of the flood unit immediately after re-entry were species of *Aspergillus* and *Penicillium* (Table 2). On the

Table 2. Spore measurements in air at reentry of flood unit 3 wk after flooding had subsided.[a]

Spore type	Spores outside of flood unit		Spores on east side of flood unit		Spores on west side of flood unit	
	Spores/m^{-3}	Percent of total	Spores/m^{-3}	Percent of total	Spores/m^{-3}	Percent of total
Alternaria	211	1.6	40	0.1	84	0
Ascospores	1060	8.2	295	0.6	127	0
Aspergillus/Penicillium	211	1.6	**51,500**	**97.5**	**341,000**	**98.8**
Basidiospores	**6540**	**50.3**	295	0.6	42	0
Bipolaris	84	0.6	13	0	42	0
Chaetomium	42	0.3	—	—	13	0
Cladosporium	**3630**	**27.9**	549	1	844	0.2
Curvularia	549	4.2	—	—	—	—
Epicoccom	338	2.6	42	0.1	—	—
Fusarium	—	—	—	—	—	—
Ganoderma	84	0.6	42	0.1	—	—
Myxomycetes	84	0.6	—	—	42	0
Pithomyces	—	—	—	—	—	—
Rust	—	—	—	—	—	—
Scopulariopsis	—	—	—	—	—	—
Stachybotrys	—	—	—	—	2360	0.7
Torula	—	—	—	—	—	—
Ulocladium	—	—	—	—	13	0
Unidentifiable spores	42	0.3	—	—	—	—
Cercospora	42	0.3	—	—	—	—
Nigrospora	42	0.3	13	0	—	—
Spegazzinia	13	0.1				
	—	—	—	—		
Total fungi	13,000	100	52,800	100	345,000	100

[a] Air samples were collected with Air-O-Cell cartridges and the volume of each sample was 75 L. Identifications were done by EMSL Analytical, Inc.[b]
[b] Bold indicates notable percentage contributions to the totals.

west side of the flood unit, 98.8% of the spores were of these types. On the east side of the flood unit, 97.5% were of these types. Fungal spores collected outside the flood unit were mostly basidiospores of unknown basidiomycetes (50.3%) or *Cladosporium* (27.9%). Basidiospores and *Cladosporium* were also observed within the flood unit but at lower levels. The total mold spore count was larger internally (345×10^3 and 528×10^2 for the west and east sides of the flood unit, respectively) than externally (13×10^3).

Drying the materials in the flood unit had a dramatic effect on the types and frequencies of mold spores (Table 3). Moisture contents decreased from 45 to 51% for wet gypsum adjacent to a stud or insulation to 2-9% for dried gypsum similarly located, from 24 to 41% for wet gypsum not located near a stud or insulation to 2-15% for dried gypsum similarly located, from 11 to 14% for wet sheathing above the water line and 26-39% for wet sheathing below the water line to 6-8% and 12-36% for dried sheathing above and below the water line, respectively, and from 100% for wet fiberglass batt insulation above the water line and 90% below the water line to 31% for dried fiberglass batt insulation above the water line and 88% for dried below the water line. Remediation and restoration continued to decrease the mold spore counts in the air (Table 4). The total amount measured inside was 177×10^2 spores/m^{-3} after drying and 79×10^2 spores/m^{-3} after remediation and restoration. In both cases, the total spore count inside was lower than the total outside spores.

Basidiomycete spores comprised the majority of total spores measured outside of the flood unit: 50.3% at re-entry, 95.3% after drying, and 85.1% after remediation. In contrast, inside the flood unit, basidiomycetes spores comprised a majority after drying (78.5%) and after remediation (89.5%), whereas at re-entry, these spore

Table 3. Spore measurements from the air inside the flood unit and outside after drying for 1 mo and before remediation.[a]

	Spores outside of flood unit		Spores inside of flood unit	
Spore type	Spores/m^{-3}	Percent of total	Spores/m^{-3}	Percent of total
Alternaria	21	0.1	21	0.1
Ascospores	401	1.7	**1480**	**8.4**
Aspergillus/*Penicillium*	84	0.4	253	1.4
Basidiospores	**22,400**	**95.3**	**13,900**	**78.5**
Bipolaris	84	0.4	422	2.4
Chaetomium	—	—	443	2.5
Cladosporium	401	1.7	1060	6
Curvularia	42	0.2	84	0.5
Epicoccom	7	0	—	—
Fusarium	—	—	—	—
Ganoderma	21	0.1	—	—
Myxomycetes	63	0.3	—	—
Pithomyces	—	—	13	0.1
Rust	—	—	—	—
Scopulariopsis	—	—	—	—
Stachybotrys	—	—	13	0.1
Torula	—	—	—	—
Ulocladium	—	—	7	0
Unidentifiable spores	—	—	—	—
Cercospora	21	0.1	—	—
Nigrospora	—	—	42	0.2
Spegazzinia	—	—	—	—
Total fungi	23,500	100	17,700	100

[a] Air samples were collected with Air-O-Cell cartridges, and the volume of each sample was 150 L. Identifications were done by EMSL Analytical, Inc.[b]
[b] Bold indicates notable percentage contributions to the totals.

types were a very small minority (0.6%) of the total. Basidiomycete spores often occur in large numbers in the air, and some researchers consider that this fungal group may be overlooked in many studies. Fröhlich-Nowoisky et al (2009) analyzed coarse and fine particulate matter for fungal species during the course of a year using DNA-based techniques. They found that basidiomycete spores comprised 64% of the yearly total, whereas the ascomycetes comprised only 34%. The ratio of basidiomycetes to ascomycetes was highest in the coarse matter in summer and fall and the ratio was lowest in the fine matter during winter and spring. These authors identified many of the basidiomycetes and a majority (87%) were in the class Agaricomycetes, which contains the wood decay fungi. In this study, only at re-entry were the inside levels of basidiospores much lower than the outside levels. This suggests that these spore types cannot remain in the air under a very high moisture environment. However, as the wood and wall materials dry and the humidity inside drops, the presence of high levels of basidiospores in the outside air provides an opportunity for wood decay fungi to invade the wall cavities and possibly become established if sufficient moisture remains in the walls.

The molds identified in this study match very closely the molds identified in post-Hurricane Katrina studies (Chew et al 2006; Solomon et al 2006; Rao et al 2007; Riggs et al 2008; Adhikari et al 2009, 2010; Bloom et al 2009). All these studies found *Aspergillus*/*Penicillium* through spore counts, culture, and/or PCR to be the predominant indoor fungi. By culture and cloning, we were able to identify four species of *Aspergillus* and two species of *Penicillium* (Table 5). We found the presence of *Penicillium* species to be much less common than the presence of *Aspergillus* species. Three of the four *Aspergillus* species (*A. fumigatus*, *A. niger*, and *A. versicolor*) and the *Penicillium* species found in our study are listed as Group 1 molds by the EPA (Vesper et al

Table 4. Spore measurements inside and outside the flood unit after remediation and restoration.[a]

	Spores outside of flood unit		Spores inside of flood unit	
Spore type	Spores/m^{-3}	Percent of total	Spores/m^{-3}	Percent of total
Alternaria	—	—	7	0.1
Ascospores	**1080**	**9.3**	**253**	**3.2**
Aspergillus/*Penicillium*	63	0.5	21	0.3
Basidiospores	**9870**	**85.1**	**7070**	**89.5**
Bipolaris	63	0.5	21	0.3
Chaetomium	—	—	21	0.3
Cladosporium	190	1.6	211	2.7
Curvularia	211	1.8	232	2.9
Epicoccom	—	—	—	—
Fusarium	—	—	—	—
Ganoderma	63	0.5	21	0.3
Myxomycetes	—	—	21	0.3
Pithomyces	7	0.1	—	—
Rust	—	—	—	—
Scopulariopsis	—	—	—	—
Stachybotrys	—	—	—	—
Torula	—	—	—	—
Ulocladium	—	—	—	—
Unidentifiable spores	—	—	—	—
Cercospora	—	—	—	—
Nigrospora	21	0.2	20	0.3
Spegazzinia	21	0.2	—	—
Total fungi	11,600	100	7900	100

[a] Air samples were taken approximately 2 mo after opening the unit. Air samples were collected with Air-O-Cell cartridges, and the volume of each sample was 150 L. Identifications were done by EMSL Analytical, Inc.[b]

[b] Bold indicates notable percentage contributions to the totals.

2007a) and are all known to produce mycotoxins (Frisvad 1989; Nielsen et al 1998b).

Material Sampling Results—Exposure to Flood Water

The interior of the housing unit showed water stains and blistering of the gypsum wallboards below the water line. Severe mold growth was observed above the water line to the ceiling (Fig 2a-b). Figure 2b shows water stains below the water line on the sheathing and wood framing inside the wall. These walls were a possible source for the mold spores in the air of the flood unit at re-entry. The fungi identified from the pond water and wall material samples, segregated by exposure to the flood water, are listed in Table 5. Although 13 different fungi were isolated and identified from the water, the water actually contained very low levels of these fungi. It was necessary to plate 500 µL of pond water straight on the culture media before fungal colonies grew. This made it easy to isolate and subculture these colonies, resulting in a relatively large number that could be identified. Of the 13 different fungi identified from the pond water, five were unique to the water and were not detected on any wall material sampled.

On the wet wall materials exposed above the water level, 14 different fungal species were identified, and 11 species were identified on the wet wall materials exposed below the flood water (Table 5). Seven of these species were shared in common between these two exposures. It would be useful to determine the potential source of the fungi growing on the wet building materials. Of the total fungi identified in this study, 30% were detected in the air samples, 23% in the water samples, 13% in both air and water, 27% in neither water nor air, and 17% in the water but not on the wall materials. Sediment, which entered with the water and was deposited on the walls, may also have been a source. Fröhlich-Nowoisky et al (2009) found

Table 5. Fungi identified from pond water and wall materials separated into exposure above the flood water line and exposure below the flood water line.[a]

Mold	Pond water	Wet materials above water line	Wet materials below water line	Dry materials above water line	Dry materials below water line
Acremonium strictum	–	X	X	–	–
Alternaria sp.	–	X	–	–	–
Aspergillus fumigatus	–	–	–	X	X
Aspergillus niger	–	X	X	X	–
Aspergillus terreus	–	–	X	–	–
Aspergillus versicolor	X	X	–	–	–
Bionectria sp.	–	X	–	–	–
Chaetomium globosum	–	X	X	–	X
Cladosporium sp.	–	–	X	–	–
Fusarium sp.	X	–	–	–	–
Fusarium oxysporum	–	X	X	X	–
Fusarium solani	–	X	–	–	–
Hexagonia hirta	–	–	–	X	–
Nectria cinnabarina	X	–	–	–	–
Paecilomyces variotii	–	–	–	X	–
Penicillium sp.	X	–	–	–	X
Penicillium purpurogenum	X	–	X	X	–
Peniophora aurantiaca	X	–	–	–	–
Pestalotiopsis/*Pestalocia* sp.	–	X	–	–	–
Phaeosphaeria avenaria	–	–	–	X	–
Phanerochaete sordida	X	–	–	–	–
Phoma herbarum	X	X	–	–	–
Phoma glomerata	X	–	–	–	–
Psathyrella sp.	X	–	–	–	–
Rhizoctonia solani	–	–	X	–	–
Stachybotrys chartarum	–	–	–	X	X
Trametes versicolor	X	X	–	–	–
Trichoderma harzianum	X	X	X	–	–
Trichoderma reesei	–	X	X	–	–
Trichoderma viride	X	X	X	–	–

[a] The flood unit was drained of pond water and left closed for 3 wk prior to wet material sampling. Dry materials were removed approximately 7 mo after the flood unit was opened. X indicates presence and – indicates absence of a species.

that 70% of fungi identified from air samples taken during the course of a year were detected in a single sample. This highlights the extreme variability and diversity of fungi that can be present in air at different times of the year. In this study, air sampling did not distinguish different species of *Aspergillus*, and *Penicillium* was grouped with the *Aspergillus*. Also, air sampling typically does not detect molds that produce spores in enclosed or sticky structures. These molds include *Chaetomium*, *Trichoderma*, and *Stachybotrys* (Samson et al 2010). This may explain why *Trichoderma* was not detected in the air samples (Samson et al 2010). In addition, the air sampling data list general groups called basidiospores and ascospores. Many of the other fungal species identified on the material samples were ascomycetes as well as one basidiomycete (*T. versicolor*).

Although more species were identified from above the water line exposure, many more fungal colonies grew on plates from material samples exposed below the water line compared with samples from above the water line. Samples from below the water line were coated with soil particles and brown water residues (Fig 2). These residues probably supported a much higher concentration of fungi both as a food source as well as a source of inoculum. It is our opinion that the flood water used in this study was similar to flood water from rivers

(a) (b)

Figure 2. View on re-entry of (a) the south wall from which samples were sent to Mississippi State University for testing of fungi and (b) the sheathing and wood framing inside the south wall after samples were removed for testing.

that are impacted by agricultural production. Pesticides, nutrients, sediments, oil, and grease were all present in this water (Aglan et al 2014) and probably influenced the types of fungi that remained after the flood. In particular, the nutrients in the water probably enhanced mold development by providing a source of nitrogen. This again points out that higher species diversity does not necessarily correlate to higher concentrations of fungi. The three species of *Trichoderma* and the *Rhizoctonia* found on the wet samples are considered to be very rapid growers and, when present, can quickly overrun a culture plate. This will both inhibit the slower-growing fungi from developing as well as visually mask smaller colonies of other fungi. There are also indications that these species were present in high concentrations primarily below the water line. Although cloning of the DNA extracted from a material sample does not quantitate the amount of a species, 100% of the clones sequenced in some samples were one of these species. This indicates there was a large amount of these species' DNA in the extraction sample. The presence of these species would probably decrease the number of other species isolated and identified and might explain why species with moderate growth rates such as *Stachybotrys* were not found on wet material samples.

The species of fungi found above and below the exposure to the flood water drastically changed after the flood unit was left to dry for approximately 7 mo. Eight species were identified from the materials exposed above the water line and only four species identified from materials exposed below the water line. At this point, the moisture content for the above and below material samples was similar (data not given). There were two species in common between the dry above-water exposure and the wet above-water exposure, *A. niger* and *Fusarium oxysporum*, and no species in common between the dry and wet below-water exposure. Two species were common to both dry material exposures, notably *A. fumigatus* and *S. chartarum.* All species found on the dry below-water exposure were also found in the air samples taken inside the flood unit after drying and before remediation (Table 3), whereas five of eight species (or groups) found on the dry above-water exposure were also in the air samples taken at that time (Table 3).

Aspergillus species were the predominant mold found in this study followed by *C. globosum* and then *Trichoderma*. Both *C. globosum* and *T. viride* are Group 1 molds. These molds grow

very rapidly, are known to produce mycotoxins, and are often associated with water-damaged buildings, water-saturated wood, and cellulose-based materials (Samson et al 2011). In this study, all the *Trichoderma* species were found only in the wet materials and were notably absent from the dry material samples. The other Group 1 molds found in this study, and often encountered in the other cited studies, were *Paecilomyces variotti* and *S. chartarum*. *Stachybotrys* has gained notoriety during the past years because of its production of six toxic macrocyclic and four noncyclic trichothecene mycotoxins (Nielsen et al 1998a). Although frequently encountered in water-damaged homes, *Stachybotrys* is not nearly as common as *Aspergillus/Penicillium* species. *Stachybotrys* is also often associated with cellulose-based building materials and high moisture content (Nikulin et al 1994; Samson et al 2011). This mold is often missed by air sampling because it does not become airborne readily and because the spores appear to lose their culturability soon after release (Miller 1992; Kildesø et al 2003; Andersen et al 2011). In this study, *Stachybotrys* was found in low levels inside the flood unit by air sampling after re-entry (Table 2) and after drying (Table 3). By culture isolations and cloning, *Stachybotrys* was only found on the materials after drying (Table 5). It is likely that *Stachybotrys* was present on some of the wet materials but was not detected. A current study is quantitating certain mold species, including *Stachybotrys*, on the different components of wet and dry building materials by quantitative PCR with the intent of mapping the presence of these mold species in the different materials.

Material Sampling Results—Comparison of Materials

Table 6 segregates the fungal species identified by type of wall material. Nine species were identified from the wet gypsum board, eight from the batt insulation, and six from the wood sheathing. The gypsum and batt shared three species in common (*Acremonium strictum, A. niger,* and *A. terreus*), whereas the gypsum and wood sheathing shared three different species in common (*C. globosum, T. reesei,* and *T. viride*). The wood sheathing and batt shared no species in common. Observations made during the culturing of these materials support these three wall material types as harboring more fungi compared with the other wall materials. Only zero to three colonies developed per plate when the vinyl siding, house wrap, and wood stud samples were plated on culture media. It is not surprising that vinyl siding and house wrap did not support fungal growth because these materials are hydrophobic. When similar-sized pieces of the gypsum papers, batt, and wood sheathing were plated, 25-100 colonies per plate developed. The batt insulation appeared to support the greatest concentration of fungi compared with all other wall materials and also contained the most moisture (90% below water line). The gypsum board was separated into the front paper, which was painted, the back paper, which was brown and uncoated, and the gypsum itself for culturing. The gypsum did not support the presence of fungi, but both paper sidings supported high levels of fungal growth. Overall, the gypsum paper ranked second highest in supporting fungal growth and also had the second highest moisture content (25-51%). The plywood sheathing also supported very large numbers of fungi. Samples taken from the sides of the plywood produced much higher numbers of fungal colonies compared with samples taken from the face veneers. The water penetrated through the sides into the plies and thus the sides probably maintained higher moisture levels (11-39%) for longer periods of time. The sheathing culture plates were dominated by the *Trichoderma* species. *Trichoderma* cultures were found on the wood sheathing, the wood stud, and the gypsum papers but not on the batt insulation. *Trichoderma* species are well-known cellulolytic fungi, and they partitioned to, and developed on, the cellulose-based wall materials.

The dry material samples contained a very different fungal population compared with their wet counterparts with only one species in common (*A. niger*) between the dry and wet gypsum

Table 6. Fungi identified from different wall materials by culture and DNA-based identifications.[a]

Mold	Wet gypsum board	Dry gypsum board	Wet batt insulation	Wet wood sheathing	Wet wood stud	Dry wood stud	Wet vinyl siding
Acremonium strictum	X	–	X	–	–	–	–
Alternaria sp.	X	–	–	–	–	–	–
Aspergillus fumigatus	–	X	–	–	–	X	–
Aspergillus niger	X	X	X	–	–	X	X
Aspergillus terreus	X	–	X	–	–	–	–
Aspergillus versicolor	–	–	X	–	–	–	–
Bionectria sp.	X	–	–	–	–	–	–
Chaetomium globosum	X	–	–	X	–	X	X
Cladosporium sp.	X	–	–	–	–	–	–
Fusarium oxysporum	–	X	X	–	–	–	–
Fusarium solani	–	–	X	–	–	–	–
Hexagonia hirta	–	X	–	–	–	–	–
Paecilomyces variotii	–	X	–	–	–	X	–
Penicillium sp.	–	X	–	–	–	–	–
Penicillium purpurogenum	–	–	–	X	–	X	–
Pestalotiopsis/*Pestalocia*	–	–	X	–	–	–	–
Phaeosphaeria avenaria	–	X	–	–	–	–	–
Phoma herbarum	–	–	X	–	–	–	–
Rhizoctonia solani	–	–	–	X	–	–	–
Stachybotrys chartarum	–	X	–	–	–	X	–
Trichoderma harzianum	–	–	–	X	–	–	–
Trichoderma reesei	X	–	–	X	X	–	–
Trichoderma viride	X	–	–	X	–	–	–

[a] The flood unit was drained of pond water and left closed for 3 wk prior to wet material sampling. Before sampling, dry materials (gypsum and wood stud only) were air-dried for approximately 7 mo after the flood unit was opened. X indicates presence and – indicates absence of a species.

board samples and no species in common between the dry and wet wood studs. Although eight species were identified on the dry gypsum and six species on the dry wood studs, these dry materials did not support a heavy concentration of these fungi. Four species were common to the dry gypsum and the dry wood studs. One of these was the toxin-producing *S. chartarum*, which was identified from the dry material samples by both cultural and cloning. Notably absent from the dry material samples were all of the *Trichoderma* species, which tended to rapidly overgrow the culture plates.

The relationship between molds and different building materials has been primarily evaluated in Denmark and Finland. Andersen et al (2011) analyzed 5300 surface samples from various building materials exposed to water damage. Principal component analysis found associations between certain species and materials. Plaster and concrete were the two materials most often affected by molds followed by wood, wallpaper, and gypsum. Gravesen et al (1999) found the building materials most vulnerable to mold were the cellulose-based materials such as wood, jute, wallpaper, and cardboard. Tuomi et al (2000) measured mycotoxin production on different water-damaged materials and found that 46% of the cellulose materials, 53% of the gypsum, and 43% of the synthetic materials sampled contained mycotoxins. In this study, the batt insulation supported the greatest concentration of fungi compared with all other wall materials followed by the paper sidings of the gypsum board and then the plywood sheathing. The solid wood studs, vinyl siding, and house wrap all supported very low concentrations of mold.

CONCLUSIONS

The flood unit clearly provided a good model to examine molds that result during flooding. The mold spore concentrations and species observed in the air and on the materials were consistent

with those expected for a flooded building. The changes in the mold spores observed on drying of the flood unit and the remediation of the flood unit indicated the effectiveness of these two procedures in ultimately producing an indoor environment that is at least representative of the outdoors. Future questions we hope to address are how different wall materials and wall construction practices influence concentrations and species of mold that develop after floods.

ACKNOWLEDGMENTS

This work was funded by the Department of Homeland Security sponsored Southeast Region Research Initiative at the Department of Energy's Oak Ridge National Laboratory (ORNL). Mr. Jeff Stringer, Lab Manager at Tuskegee University, is acknowledged for performing the experiment and collecting the air and mold samples. The guidance and support of Dr. Benjamin Thomas of ORNL is greatly appreciated. This manuscript has been approved by the Forest and Wildlife Research Center, Mississippi State University as Article FP737.

REFERENCES

Adhikari A, Jung J, Reponen T, Lewis JS, DeGrasse EC, Grimsley LF, Chew GL, Grinshpun SA (2009) Aerosolization of fungi, (1-3)-β-D glucan, and endotoxin from flood-affected materials collected in New Orleans homes. Environ Res 109:215-224.

Adhikari A, Lewis JS, Reponen T, DeGrasse EC, Grimsley LF, Chew GL, Iossifova Y, Grinshpun SA (2010) Exposure matrices of endotoxin, (1-3)-β-D-glucan, fungi, and dust mite allergens in flood-affected homes of New Orleans. Sci Total Environ 408:5489-5498.

Aglan H, Ludwick A, Borazjani H, Kitchens, S, Diehl S, Amburgey T (2014) Diffusion characteristics in building components of immiscible hydrocarbon–water mix from flooding (In preparation).

Aglan H, Ludwick A, Kitchens S, Amburgey T, Diehl S, Borazjani H (2013) Effect of long-term exposure and delayed drying time on moisture and mechanical integrity of flooded homes. J Flood Risk Mgmt (doi:10.1111/jfr3.12062).

Andersen B, Frisvad JC, Søndergaard I, Rasmussen IS, Larsen LS (2011) Associations between fungal species and water-damaged building materials. Appl Environ Microbiol 77:4180-4188.

Barbeau DN, Grimsley LF, White LE, El-Dahr JM, Lictveld M (2010) Mold exposure and health effects following hurricanes Katrina and Rita. Annu Rev Public Health 3:165-178.

Bloom E, Grimsley LF, Pehrson C, Lewis J, Larsson L (2009) Molds and mycotoxins in dust from water-damaged homes in New Orleans after hurricane Katrina. Indoor Air 19:153-158.

Brandt M, Brown C, Burkhart J, Burton N, Cox-Ganser J, Damon S, Falk H, Fridkin S, Garbe P, McGeehin M, Morgan J, Page E, Rao C, Redd S, Sinks T, Trout D, Wallingford K, Warnock D, Weissman D (2006) Mold prevention strategies and possible health effects in the aftermath of hurricanes and major floods. Morb Mortal Wkly Rep 55(RR-8):1-27.

Cabral JPS (2010) Can we use indoor fungi as bioindicators of indoor air quality? Historical perspectives and open questions. Sci Total Environ 408:4285-4295.

Chew GL, Wilson J, Rabito FA, Grimsley F, Iqbal S, Reponen T, Muilenberg ML, Thorne PS, Dearborn DG, Morley RL (2006) Mold and endotoxin levels in the aftermath of Hurricane Katrina: A pilot project of homes in New Orleans undergoing renovation. Environ Health Perspect 114:1883-1889.

EMSL (2012) Air-O-Cell sampling guide. http://www.emsl.com/index.cfm?nav=Sampling_Guides&action=show&SamplingGuideID=9 (accessed 3 April 2012).

Fisk WJ, Lei-Gomez Q, Mendell MJ (2007) Meta-analyses of the associations of respiratory health effects with dampness and mold in homes. Indoor Air 17:284-296.

Flannigan B, Miller JD (2011) Microbial growth in indoor environments. Pages 57-107 *in* B Flannigan, RA Samson, and JD Miller, eds. Microorganisms in homes and indoor work environments. CRC Press, Boca Raton, FL.

Frisvad JC (1989) The connection between the *Penicillia* and *Aspergilli* and mycotoxins with special emphasis on misidentified isolates. Arch Environ Contam Toxicol 18:452-467.

Fröhlich-Nowoisky J, Pickersgill DA, Després VR, Pöschl U (2009) High diversity of fungi in air particulate matter. Proc Natl Acad Sci USA 106:12814-12819.

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity to basidiomycetes—Applications to the identification of mycorrihizae and rusts. Mol Ecol 2:113-118.

Gravesen S, Nielsen PA, Iversen R, Neilsen KF (1999) Microfungal contamination of damp buildings—Example of risk constructions and risk materials. Environ Health Perspect 107:505-508.

Kildesø J, Würtz H, Nielsen KF, Kruse P, Wilkins K, Thrane U, Gravesen S, Neilsen PA, Schneider T (2003) Determination of fungal spore release from wet building materials. Indoor Air 13:148-155.

Koskinen OM, Husman TM, Meklin TM, Nevalainen AI (1999) The relationship between moisture or mould observations in houses and the state of health of their occupants. Eur Respir J 14:1363-1367.

Miller JD (1992) Fungi as contaminants in indoor air. Atmos Environ 26A:2163-2172.

Miller JD (2011) Mycological investigations of indoor environments. Pages 229-245 *in* B Flannigan, RA Samson, JD Miller, eds. Microorganisms in homes and indoor work environments. CRC Press, Boca Raton, FL.

Mudarri D, Fisk WJ (2007) Public health and economic impact of dampness and mold. Indoor Air 17:226-235.

NAS (2004) Damp indoor spaces and health. National Academy of Sciences Committee on Damp Indoor Spaces and Health, National Academy Press, Washington, DC. 370 pp.

NAS (2013) Abrupt impacts of climate change: Anticipating surprises. National Academy of Sciences Committee on Understanding and Monitoring Abrupt Climate Change and its Impacts. National Academy Press, Washington, DC. 250 pp.

Nielsen KF, Hansen MØ, Larsen TO, Thrane U (1998a) Production of trichothecene mycotoxins on water damaged gypsum boards in Danish buildings. Int Biodeter Biodegr 42:1-7.

Nielsen KF, Thrane U, Larsen TO, Nielsen PA, Gravesen S (1998b) Production of mycotoxins on artificially inoculated building materials. Int Biodeter Biodegr 42:9-16.

Niemeier RT, Sivasubramani SK, Reponen T, Grinshpun SA (2006) Assessment of fungal contamination in moldy homes: Comparison of different methods. J Occup Environ Hyg 3:262-273.

Nikulin M, Pasanen A-L, Berg S, Hintikka E-L (1994) *Stachybotrys atra* growth and toxin production in some building materials and fodder under different relative humidities. Appl Environ Microbiol 60:3421-3424.

Park J-H, Schleiff PL, Attfield MD, Cox-Ganser JM, Kreiss K (2004) Building-related respiratory symptoms can be predicted with semi-quantitative indices of exposure to dampness and mold. Indoor Air 14:425-433.

Pirie RS, Collie DD, Dixon PM, McGorum BC (2003) Inhaled endotoxin and organic dust particulates have synergistic proinflammatory effects in equine heaves (organic dust-induced asthma). Clin Exp Allergy 33: 676-683.

Prezant B, Weekes D, Miller JD (2008) Recognition, evaluation and control of indoor mold. American Industrial Hygiene Association, Fairfax, VA. 253 pp.

Rao CY, Riggs MA, Chew GL, Muilenberg ML, Thorne PS, Van Sickle D, Dunn KH, Brown C (2007) Characterization of airborne molds, endotoxins, and glucans in homes in New Orleans after Hurricanes Katrina and Rita. Appl Environ Microbiol 73:1630-1634.

Riggs MA, Rao CY, Brown CM, Van Sickle D, Cummings KJ, Dunn KH, Deddens JA, Ferdinands J, Callahan D, Moolenaar RL, Pinkerton LE (2008) Resident cleanup activities, characteristics of flood-damaged homes and airborne microbial concentrations in New Orleans, Louisiana, October 2005. Environ Res 106:401-409.

Rylander R, Norrhall M, Engdahl U, Tunsater A, Holt PG (1998) Airways inflammation, atopy, and (1-3)-β-D-glucan exposures in two schools. Am J Resp Crit Care 158: 1685-1687.

Samson RA, Houbraken J, Summerbell RC, Flannigan B, Miller DJ (2011) Common and important species of fungi and actinomycetes in indoor environments. Pages 321-513 *in* B Flannigan, RA Samson, JD Miller, eds. Microorganisms in homes and indoor work environments. CRC Press, Boca Raton, FL.

Samson RA, Houbraken J, Thrane U, Frisvad JC, Andersen B (2010) Food and indoor fungi. CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands. 390 pp.

Solomon GM, Hjelmroos-Koski M, Rotkin-Ellman M, Hammond SK (2006) Airborne mold and endotoxin concentrations in New Orleans, Louisiana, after flooding, October through November 2005. Environ Health Perspect 114:1381-1386.

Tuomi T, Reijula K, Johnsson T, Hemminki K, Hintikka E-L, Lindroos O, Kalso S, Koukila-Kähkölä P, Mussalo-Rauhamaa H, Haahtela T (2000) Mycotoxins in crude building materials from water-damaged homes. Appl Environ Microbiol 66:1899-1904.

Vesper S, McKinstry C, Haugland R, Wymer L, Bradham K, Ashley P, Cox D, Dewalt G, Friedman W (2007a) Development of an environmental relative moldiness index for US homes. J Occup Environ Med 49:829-833.

Vesper SJ, McKinstry C, Haugland RA, Iossifova Y, LeMasters G, Levin L, Khurana Hershey GK, Villareal M, Bernstein DI, Lockey J, Reponen T (2007b) Relative moldiness index as predictor of childhood respiratory illness. J Expo Anal Environ Epidemiol 17:88-94.

White TJ, Bruns SL, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315-322 *in* MA Innis, DH Gelfand, JJ Sninsky, and TJ White, eds. PCR protocols: A guide to methods and applications. Academic Press, San Diego, CA.