Mississippi State University
Scholars Junction

Theses and Dissertations | Theses and Dissertations

1-1-2012

Fungal Communities on Flooded Building Materials

Frederick Skrobot

Follow this and additional works at: https://scholarsjunction.msstate.edu/td

Recommended Citation
Skrobot, Frederick, "Fungal Communities on Flooded Building Materials" (2012). *Theses and Dissertations*. 2356.
https://scholarsjunction.msstate.edu/td/2356

This Graduate Thesis - Open Access is brought to you for free and open access by the Theses and Dissertations at Scholars Junction. It has been accepted for inclusion in Theses and Dissertations by an authorized administrator of Scholars Junction. For more information, please contact scholcomm@msstate.libanswers.com.

المنارة للاستشارات

www.manaraa.com

Fungal communities on flooded building materials

By

Frederick Skrobot III

A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in Forest Products
in the Department of Forest Products

Mississippi State, Mississippi

December 2012

المنارة للاستشارات

www.manaraa.com

Copyright by

Frederick Skrobot III

2012

المنارة للاستشارات

www.manaraa.com

Fungal communities on flooded building materials

By

Frederick Skrobot III

Approved:

Susan Diehl Professor of Forest Products (Director of Thesis)	Hamid Borazjani Professor of Forest Products (Committee Member)
Shane Kitchens Assistant Professor of Forest Products (Committee Member)	Tor P. Schultz Professor of Forest Products (Graduate Coordinator)
George Hopper Dean, College of Forest Resources	

www.manaraa.com

Name: Frederick Skrobot III

Date of Degree: December 15, 2012

Institution: Mississippi State University

Major Field: Forest Products

Director of Thesis: Dr. Susan V. Diehl

Title of Study: Fungal communities on flooded building materials

Pages in Study: 188

Candidate for Degree of Master of Science

Flood waters will penetrate the wall cavities of a home and the wall materials then serve as a substrate for mold development. This study measured the effect of flooding and subsequent drying on the extent and type of mold on different residential wall materials. Wet and dry wall samples were analyzed by cloning and sequencing and twenty-one mold species were identified from above and below the water line. Real-time PCR quantitated selected species on fiberglass batt insulation, gypsum wallboard, wood stud, plywood panels, vinyl siding, and house wrap. The mold species found in the highest concentration were *Aspergillus fumigatus*, *Paecilomyces variotii, Chaetomium globosum,* and *Stachybotrys chartarum*. The batt insulation supported the highest concentration of mold, followed by the wood stud, sheathing and gypsum wallboard. The high level of *Aspergillus fumigatus* and *Stachybotrys chartarum* on the wall materials seven months after flooding is a cause for concern.

www.manaraa.com

ACKNOWLEDGEMENTS

The author would like to express his deepest appreciation to Dr. Susan V. Diehl for her constant guidance, assistance, and mentoring throughout this investigation. Expressed gratitude is extended to Dr. Shane Kitchens and Dr. Hamid Borazjani for their suggestions and for serving as members of my graduate committee. Special thanks are expressed to Dr. Juliet Tang for her advice and technical assistance throughout the course of the study.

I would like to thank Tuskegee University for providing the samples and funding provided by SERRI. Additional gratitude is extended to Mary Kate Mohammadi for her laboratory assistance.

I am also appreciative of my Mom and Dad for their encouragement throughout my years of study.

المنارة للاستشارات
www.manaraa.com

TABLE OF CONTENTS

ACKNOWLEDGEMENTS ii

LIST OF TABLES v

LIST OF FIGURES viii

CHAPTER

I. INTRODUCTION 1

Introduction 1
Characteristics of Mold 2
Molds and Indoor Air Quality 4
Wall Construction 7
Identification of Mold 13
Mold, Moisture and Building Materials 19
Overview of Research 20

II. MATERIALS AND METHODS 21

Background 21
Preparation of Building Materials 29
DNA Extraction 29
Polymerase Chain Reaction (PCR) Amplification 31
Direct Purification of PCR product 32
Media for Cloning 33
Cloning Procedure 33
Plasmid Extraction 34
Enzyme Digest 35
Sequencing 35
Real-Time PCR Procedures 37
Primer Design 37
Real-Time qPCR 38
Statistical Analysis 39

III. RESULTS AND DISCUSSIONS 40

Isolation and Identification of Mold from Building Materials 40
Real-Time PCR 44

المنارة للاستشارات
www.manaraa.com

Aspergillus fumigatus..........45
Aspergillus niger..........63
Aspergillus terreus..........80
Aspergillus versicolor..........98
Chaetomium globosum..........117
Paecilomyces variotii..........133
Stachybotrys chartarum..........150
Composition of Materials..........166
Quantization of Mold on Wet Materials..........166
Quantization of Mold on Dry Materials..........175

IV. CONCLUSIONS..........177

Identification of Molds..........177
Quantiation of Molds on Wall Materials..........178
Summary of Mold Species found on Flooded Wall Materials..........179
Summary of Flooded Wall Materials..........181

REFERENCES..........184

المنارة للاستشارات
www.manaraa.com

LIST OF TABLES

2.1 Wet Building Materials Obtained from Flood Unit. 24

2.2 Dry Building Materials Obtained from Flood Unit...28

3.1 Mold species identified from wet and dry building materials. (+ means present and – means not present). 41

3.2 Mold identified from wall materials separated into exposure above the flood water line and exposure below the flood water line.(+ means present and – means not present) 43

3.3 Real-time PCR results of *Aspergillus fumigatus* DNA in nannograms per weight of material 45

3.4 Statistical analysis of each wet building material. (Means with same letter are not significantly different)...51

3.5 The average of *Aspergillus fumigatus* on both above and below the water line on wet building materials. 59

3.6 The significant difference of *Aspergillus fumigatus* on the dry building materials. (Means with the same letter are not significantly different)..60

3.7 Real-time PCR results of *Aspergillus niger* DNA in nannograms per weight of material...64

3.8 Statistical (ANOVA) anaylsis of the significant difference of dry building materials. (Means with the same letter are not significantly different)...69

3.9 Statistical analysis on *Aspergillus niger* both above and below the water line on wet materials. 76

3.10 Statistical (ANOVA) anaylsis of the significant difference of dry building materials. (Means with the same letter are not significantly different)...78

www.manaraa.com

3.11 Real-time PCR results of *Aspergillus terreus* DNA in nannograms per weight of material....81

3.12 Statistical (ANOVA) anaylsis of the significant difference of dry building materials. (Means with the same letter are not significantly different)....87

3.13 Statistical analysis on *Aspergillus terreus* both above and below the water line on wet materials....95

3.14 Statistical (ANOVA) anaylsis of the significant difference of dry building materials. (Means with the same letter are not significantly different)....96

3.15 Real-time PCR results of *Aspergillus versicolor* DNA in nannograms per weight of material 99

3.16 Statistical (ANOVA) anaylsis of the significant difference of wet building materials. (Means with the same letter are not significantly different)....105

3.17 Statistical analysis on *Aspergillus versicolor* both above and below the water line on all wet materials....113

3.18 Statistical (ANOVA) anaylsis of the significant difference of dry building materials. (Means with the same letter are not significantly different)....114

3.19 Real-time PCR results of *Chaetomium globosum* DNA in nannograms per weight of material 117

3.20 Statistical (ANOVA) anaylsis of the significant difference of wet building materials. (Means with the same letter are not significantly different)....123

3.21 Statistical analysis on *Chaetomium globosum* both above and below the water line on all wet materials....130

3.22 Statistical (ANOVA) anaylsis of the significant difference of dry building materials. (Means with the same letter are not significantly different)....131

3.23 Real-time PCR results of *Paecilomyces variotii* DNA in nannograms per weight of material 133

المنارة للاستشارات
www.manaraa.com

3.24 Statistical (ANOVA) analysis of the significant difference of dry building materials. (Means with the same letter are not significantly different).........139

3.25 Statistical analysis on *Paecilomyces variotii* both above and below the water line on all wet materials.........147

3.26 Statistical (ANOVA) anaylsis of the significant difference of dry building materials. (Means with the same letter are not significantly different).........148

3.27 Real-time PCR results of *Stachybotrys chartarum* DNA in nannograms per weight of material 151

3.28 Statistical (ANOVA) anaylsis of the significant difference of wet building materials. (Means with the same letter are not significantly different).........157

3.29 Statistical analysis on *Stachybotrys chartarum* both above and below the water line on the wet materials.........162

3.30 Statistical (ANOVA) anaylsis of the significant difference of dry building materials. (Means with the same letter are not significantly different).........164

المنارة للاستشارات
www.manaraa.com

LIST OF FIGURES

1.1 Diagram of building materials used for wall construction. 11

1.2 Flood Unit testing site at Tuskegee University (Tuskegee, AL). Photograph Courtesy of Tuskegee University. 22

1.3 Flood Unit opened after three weeks showing mold development. Photograph Courtesy of Tuskegee University. 23

1.4 The different sections of gypsum wallboard 24

3.1 The distribution of *Aspergillus fumigatus*, based on averaged DNA concentrations, on the different wet building materials. Samples with a 7 came from the same wall unit..50

3.2 The distribution of *Aspergillus fumigatus*, based on DNA concentrations, on the different components of the batt insulation-7. 52

3.3 The distribution of *Aspergillus fumigatus*, based on DNA concentrations, on the different components of gypsum-1. 53

3.4 The distribution of *Aspergillus fumigatus*, based on DNA concentrations, on the different components of gypsum-2. 54

3.5 The distribution of *Aspergillus fumigatus*, based on DNA concentrations, on the different components of gypsum-3. 55

3.6 The distribution of *Aspergillus fumigatus*, based on DNA concentrations, on the different components of gypsum-7. 56

3.7 The distribution of *Aspergillus fumigatus*, based on DNA concentrations, on the different components of wood stud-7. 57

3.8 The distribution of *Aspergillus fumigatus*, based on DNA concentrations, on the different components of the wood sheathing, plus the house wrap, and vinyl siding-7................................58

المنارة للاستشارات

www.manaraa.com

3.9 The distribution of *Aspergillus fumigatus*, based on DNA concentrations, on the different components of the dry wall material samples. 59

3.10 The distribution of *Aspergillus fumigatus*, based on DNA concentrations, on the different components of the dry gypsum. 61

3.11 The distribution of *Aspergillus fumigatus*, based on DNA concentrations, on the different components of the dry wood stud. 62

3.12 The distribution of *Aspergillus niger*, based on averaged DNA concentrations, on the different wet building materials. Samples with a 7 came from the same wall unit. 68

3.13 The distribution of *Aspergillus niger*, based on DNA concentrations, on the different components of batt insulation-7. 70

3.14 The distribution of *Aspergillus niger*, based on DNA concentrations, on the different components of gypsum-1 71

3.15 The distribution of *Aspergillus niger*, based on DNA concentrations, on the different components of gypsum-2. 72

3.16 The distribution of *Aspergillus niger*, based on DNA concentrations, on the different components of gypsum-3. 73

3.17 The distribution of *Aspergillus niger*, based on DNA concentrations, on the different components of the gypsum-7. 74

3.18 The distribution of *Aspergillus niger*, based on DNA concentrations, on the different components of the wood stud-7. 75

3.19 The distribution of *Aspergillus niger*, based on DNA concentrations, on the different components of the wet wood sheathing, plus the house wrap, and vinyl siding-7. 76

3.20 The distribution of *Aspergillus niger*, based on DNA concentrations, on the different components of dry building materials. 77

3.21 The distribution of *Aspergillus niger*, based on DNA concentrations, on the different components of the dry wood stud. 78

3.22 The distribution of *Aspergillus niger*, based on DNA concentrations, on the different components of the dry gypsum. 79

المنارة للاستشارات
www.manaraa.com

3.23 The distribution of *Aspergillus terreus*, based on averaged DNA concentrations, on the different wet building materials. Samples with a 7 came from the same wall unit...86

3.24 The distribution of *Aspergillus terreus*, based on DNA concentrations, on the different components of batt insulation-7. 88

3.25 The distribution of *Aspergillus terreus*, based on averaged DNA concentrations, on the different components of wet gypsum-1.............89

3.26 The distribution of *Aspergillus terreus*, based on DNA concentrations, on the different components of the wet Gypsum-2. 90

3.27 The distribution of *Aspergillus terreus*, based on DNA concentrations, on the different components of wet gypsum-3. 91

3.28 The distribution of *Aspergillus terreus*, based on DNA concentrations, on the different wet components of gypsum-7. 92

3.29 The distribution of *Aspergillus terreus*, based on DNA concentrations, on the different components of the wet wood stud-7. 93

3.30 The distribution of *Aspergillus terreus*, based on DNA concentrations, on the different components of wet wood sheathing, plus the house wrap, and vinyl siding-7. 94

3.31 The distribution of *Aspergillus terreus*, based on DNA concentrations, on the different components of the dry building materials. 95

3.32 The distribution of *Aspergillus terreus*, based on DNA concentrations, on the different section of the dry wood stud. 96

3.33 The distribution of *Aspergillus terreus*, based on DNA concentrations, on the different components of the dry gypsum....................................97

3.34 The distribution of *Aspergillus versicolor*, based on averaged DNA concentrations, on the different wet building materials. Samples with a 7 came from the same wall unit..104

3.35 The distribution of *Aspergillus versicolor*, based on DNA concentrations, on the different components of the wet batt insulation-7...106

3.36 The distribution of *Aspergillus versicolor*, based on DNA concentrations, on the different components of the gypsum-1. 107

المنارة للاستشارات
www.manaraa.com

3.37 The distribution of *Aspergillus versicolor*, based on DNA concentrations, on the different components of wet gypsum-2............108

3.38 The distribution of *Aspergillus versicolor*, based on DNA concentrations, on the different components of the wet gypsum-3...109

3.39 The distribution of *Aspergillus versicolor*, based on DNA concentrations, on the different components of the wet gypsum-7...110

3.40 The distribution of *Aspergillus versicolor*, based on DNA concentrations, on the different components of the wet wood stud-7...111

3.41 The distribution of *Aspergillus versicolor*, based on DNA concentrations, on the different components for the wet wood sheathing, plus house wrap, and vinyl siding-7 building. 112

3.42 The distribution of *Aspergillus versicolor*, based on DNA concentrations, on the different dry building materials........................113

3.43 The distribution of *Aspergillus versicolor*, based on DNA concentrations, on the different components of the dry wood stud. 115

3.44 The distribution of *Aspergillus versicolor*, based on DNA concentrations, on the different components of the dry gypsum. 116

3.45 The distribution of *Chaetomium globosum*, based on averaged DNA concentrations, on the different wet building materials. Samples with a 7 came from the same wall unit..122

3.46 The distribution of *Chaetomium globosum*, based on DNA concentrations, on the different components of the wet batt insulation-7...124

3.47 The distribution of *Chaetomium globosum*, based on DNA concentrations, on the different components of the wet gypsum-1...125

3.48 The distribution of *Chaetomium globosum*, based on DNA concentrations, on the different components of the wet gypsum-2...126

المنارة للاستشارات
www.manaraa.com

3.49 The distribution of *Chaetomium globosum*, based on DNA concentrations, on the different components of the wet gypsum-3 material. 127

3.50 The distribution of *Chaetomium globosum*, based on DNA concentrations, on the different components of the wet gypsum-7. 127

3.51 The distribution of *Chaetomium globosum*, based on DNA concentrations, on the different components of the wet wood stud-7. 128

3.52 The distribution of *Chaetomium globosum*, based on DNA concentrations, on the wet wood sheathing, plus the house wrap, and vinyl siding-7 materials. 129

3.53 The distribution of *Chaetomium globosum*, based on DNA concentrations, on the different the dry building materials. 130

3.54 The distribution of *Chaetomium globosum*, based on DNA concentrations, on the different components of the dry wood stud. 131

3.55 The distribution of *Chaetomium globosum*, based on DNA concentrations, on the different components of the dry gypsum. 132

3.56 The distribution of *Paecilomyces variotii*, based on averaged DNA concentrations, on the different wet building materials. Samples with a 7 came from the same wall unit. 138

3.57 The distribution of *Paecilomyces variotii*, based on DNA concentrations, on the different components of batt insulation-7. 140

3.58 The distribution of *Paecilomyces variotii*, based on DNA concentrations, on the different components of the wet gypsum-1. 141

3.59 The distribution of *Paecilomyces variotii*, based on DNA concentrations, on the different components of the wet gypsum-2. 142

3.60 The distribution of *Paecilomyces variotii*, based on DNA concentrations, on the different components of the wet gypsum-3. 143

المنارة للاستشارات
www.manaraa.com

3.61 The distribution of *Paecilomyces variotii*, based on DNA concentrations, on the different components of the wet gypsum-7. 144

3.62 The distribution of *Paecilomyces variotii*, based on DNA concentrations, on the different components of the wet wood stud-7. 145

3.63 The distribution of *Paecilomyces variotii*, based on DNA concentrations, on the different components of wood sheathing, plus house wrap, and vinyl siding-7. 146

3.64 The distribution of *Paecilomyces variotii*, based on DNA concentrations, on the different sections of the dry building materials. 147

3.65 The distribution of *Paecilomyces variotii*, based on DNA concentrations, on the different components of the dry wood stud. 148

3.66 The distribution of *Paecilomyces variotii*, based on DNA concentrations, on the different components of the dry gypsum. 149

3.67 The distribution of *Stachybotrys chartarum*, based on averaged DNA concentrations, on the different wet building materials. Samples with a 7 came from the same wall unit. 156

3.68 The distribution of *Stachybotrys chartarum*, based on DNA concentrations, on the different components of the wet batt insulation-7. 158

3.69 The distribution of *Stachybotrys chartarum*, based on DNA concentrations, on the different components of the dry gypsum-1. 159

3.70 The distribution of *Stachybotrys chartarum*, based on DNA concentrations, on the different sections of the wet gypsum-2. 159

3.71 The distribution of *Stachybotrys chartarum*, based on DNA concentrations, on the different components of the wet gypsum-3. 160

3.72 The distribution of *Stachybotrys chartarum*, based on DNA concentrations, on the different components of the gypsum-7. 161

المنارة للاستشارات
www.manaraa.com

3.73 The distribution of *Stachybotrys chartarum*, based on DNA concentrations, on the different components of the wet wood sheathing, plus house wrap, and vinyl siding-7. 162

3.74 The distribution of *Stachybotrys chartarum*, based on DNA concentrations, on the different dry building materials........................163

3.75 The distribution of *Stachybotrys chartarum*, based on DNA concentrations, on the different components of the dry wood stud. 164

3.76 The distribution of *Stachybotrys chartarum*, based on DNA concentrations, on the different components of the dry gypsum. 165

3.77 The distribution of the selected mold species, based on total DNA concentrations, on the batt insulation-7...166

3.78 The distribution of the selected mold species, based on total DNA concentrations, on the gypsum-1..167

3.79 The distribution of the selected mold species, based on total DNA concentrations, on the gypsum-2 material...168

3.80 The distribution of the selected mold species, based on total DNA concentrations, on the gypsum-3..169

3.81 The distribution of the selected mold species, based on total DNA concentrations, on the gypsum-7 material...170

3.82 The distribution of the selected mold species, based on total DNA concentrations, on wood stud-7..171

3.83 The distribution of the selected mold species, based on total DNA concentrations, on wood sheathing 172

3.84 The distribution of the selected mold species, based on total DNA concentrations, on vinyl siding...173

3.85 The distribution of the selected mold species, based on toal DNA concentrations, on house wrap. 174

3.86 The distribution of the selected mold species, based on total DNA concentrations, on the dry wood stud..175

3.87 The distribution of the selected mold species, based on total DNA concentrations, on the dry gypsum...176

المنارة للاستشارات
www.manaraa.com

CHAPTER I

INTRODUCTION

Introduction

Floods are a condition that occurs when water overflows a body of water such as a stream or river caused by excess precipitation and snow melt or accumulation by drainage over low-lying areas (Du et al. 2010). It has been estimated that due to breeches of the levee system that occurred during Hurricane Katrina, 80 percent of New Orleans, Louisiana was significantly impacted by flooding (Rao et al. 2007). Floods are one of the greatest natural hazard threats to the property and safety of human communities in the United States (Brody et al. 2007). In the last decade of the 20th century, floods killed about 100,000 persons and affected over 1.4 billion people (Jonkman 2005). Floods destroy drainage systems and cause toxic materials such as paints, pesticides, and gasoline to be released into the environment. Several studies including (Brody et al. 2007) state that floods are caused mainly by the increasing developments of residential, commercial and tourism, which can decrease the run-off of water. The constant changes to the environment such as converting agricultural and forest lands to development communities, can affect the hydrological systems ability to store water. Therefore greater surface runoff volume often results in increased frequency and severity of flooding (Brody et al. 2007).

المنارة للاستشارات
www.manaraa.com

In addition to containing contaminants, flood waters can also damage building materials such as gypsum wallboard, wood, cement, and insulation allowing the growth of threats such as mold. Just an inch of flood water could cause problems for a homeowner because of the threat of potential harmful microorganisms. According to Pearce et al. (1995) flood waters cause problems for homeowners by damaging homes, depositing dirt and debris, and ruining furnishings inside the home. These building materials are prone to water damage because water can penetrate through small openings or envelopes. Once compromised by water, the building materials become a suitable environment for different microorganisms to grow.

Characteristics of Mold

Molds are fungi which are simple aerobic organisms that thrive anywhere there is oxygen, moist environments, and nutrients present. The main characteristics of mold include: 1) Rapid sporulation, 2) Heavy sporulation, 3) Growth is faster in warm climates and high humidity levels, and 4) Spore dispersal in a variety of different weather conditions. Fungi are ubiquitous in nature and can be isolated from most environments. Different fungi can survive in various temperatures ranging from psycrophiles (tolerant of cold) to thermophilic organisms that can survive in 55°C. The production of spores allows the fungi to replicate and utilize the air currents to spread to other areas (Allsop 1985). Gover (1999) describes the dispersal of fungi as an important reproductive function, in order to maintain the species, extend the existing habitat range, and to spread genetic variability.

Spores can consist of single cells or be compound structures of several cells (Allsop 1985). The spore adheres to a surface, which enables the fungus to germinate and

المنارة للاستشارات
www.manaraa.com

produce germ tubes. Germination of spores begins with the swelling of the spore due to the intake of water and metabolic products. After respiration, the spore continues to swell, and a new wall layer is formed inside the existing spore wall (Gover 1999). Once the new wall layer is formed, a germ tube penetrates as a papilla through a pore. Finally, the wall of the germ tube arises as an extension of the newly formed wall of the spore and gives rise to a mycelium (Gover 1999). Once the germ tubes start to branch and develop into hyphae, the mold is able to digest material from the environment. A spore is considered to have germinated when the length of the longest germ tube is greater than or equal to the greatest dimension of the swollen spore (Dantigny et al. 2006). Mold spores are unique because of their ability to either germinate or remain dormant even when suitable environmental conditions are present. Dormancy provides a further dispersal method because the mechanism allows spores to stay viable for an extended duration (Gover 1999). Dormancy of mold spores may discontinue once the spore encounters different environmental conditions. The micro-climate is defined from a number of environmental parameters, the first key one being the availability of water (Allsop 1985).

Mold growth is one of the first signs of biological growth linked to high moisture content in structures (Viitanen and Ojanen 2007). Moisture is the most important environmental factor that determines the growth of mold; however the materials that make up the building section also provide suitable nutrient sources to allow growth to occur. The changes in temperature and the development of condensation can also provide suitable environmental conditions for mold spores to germinate. Once the water comes in contact with the building material, the water can be drawn in through pores or capillaries by a process called absorption (Straube 2006). Most building materials are hydrophilic,

المنارة للاستشارات
www.manaraa.com

which allows materials such as gypsum or insulation, to hold water within its pores by capillary action. Once the water is drawn in by absorption, the building material becomes a suitable environment for different types of mold depending on water activity. Nutrients in the building material can provide a food source for the growth of mold. Once the building material becomes compromised by moisture, the composition of wood products allow mold to attach, germinate, and produce spores. Viitanen and Ojanen (2007) state that mold species tend to grow when the ambient relative humidity is in the range of 75-80 percent.

Molds and Indoor Air Quality

The first documented observation of ill effects associated with indoor environments is found in the Bible, in the book of Leviticus of the Old Testament (The Bible; Rylander 1998). The Environmental Protection Agency in 2001 reported that indoor air can be two to five times more polluted than outdoor air (Portnoy et al. 2005). Fungal growth in damp or water-damaged buildings worldwide is an increasing problem, which has adverse effects on both the occupants and the buildings (Anderson et al. 2011). New construction practices that reduce ventilation and inclusion of greater amounts of synthetic materials in homes may be contributing to health related illnesses (Portnoy et al. 2005). Koskinen et al. (1999) states that moisture accumulation often promotes microbial growth in structures and finished products, which leads to particulate and gaseous emissions into indoor air. Extensive studies have been conducted to test the connection between damp building materials and human health concerns linked with mold inside homes and workplaces (Curtis et al., 2004). It has been discovered that people living and working in damp or moldy buildings have increased adverse health problems due to the

المنارة للاستشارات
www.manaraa.com

toxins and other by-products produced by the mold. The types of by-products produced by mold includes β-1, 3-D-glucan (polyglucose polymers found in fungal cell walls), mycotoxins, volatile organic compounds (such as alcohols, aldehydes, and ketones), and allergens (Jarvis and Morey 2001). Mycotoxins are defined as toxic chemicals that are produced by some mold species. Mycotoxins are usually located either on mold spores or hyphal fragments.

Buildings that are susceptible to moisture provide surfaces for growth and sporulation of common molds such as, *Cladosporium sp., Penicillium sp., Fusarium sp.,* and *Aspergillus sp*, and may cause respiratory problems for humans (Portnoy et al. 2004). Mold spores can enter the body by inhalation into the lungs and cause adverse human health effects through different types of mechanisms. These mechanisms include generation of a harmful immune response such as allergy or hypersensitivity pneumonitis, direct infection by the organism, and toxic-irritant effects from mold by-products (Bush et al. 2006). If mycotoxins are present in sufficient levels, the toxigenic spores could strongly affect alveolar macrophage function posing a threat to those exposed. This may affect the physical defense mechanisms of the respiratory tract, decreasing the ability of the airways to clear contaminants (Menetrez 2009). Some mycotoxins have been known to interfere with transcription in RNA synthesis and cause breaks in DNA strands after metabolism (Rylander 1998). Due to the cytotoxic effect of mycotoxins, increased susceptibility to cancer because of prolonged exposures may also occur (Ammann 2003).

Several investigators have tested different fungal isolates from water-damaged building materials and indoor air for production of mycotoxins. It has been suggested that

المنارة للاستشارات
www.manaraa.com

the production of mycotoxins depends on the material, water activity, temperature, and other environmental factors in which molds grow (Nielson et al. 1999). Several common fungal species known for mycotoxin production on contaminated building materials are:

- *Stachybotrys chartarum*, which produces the extremely toxic macrocyclic trichothecenes, satratoxins H and G, roridin E, and verrucarins J and B on building materials (Nielson et al.1999)
- *Aspergillus versicolor,* which produces sterigmatocystin and 5-methoxysterigmatocystin on wall papered gypsum board (Nielson et al. 1999)
- *Alternaria alternata* which produces alternariol and alternariol monomethyl ether on cellulosic acoustic ceiling tiles (Ren et al. 1998)

Some species of fungi have the potential to cause the production of IgE (Immunoglobulin E) antibodies within an individual. Once IgE is produced, the individual becomes hypersensitive due to the exposure of the fungal species. Most types of fungi prefer to grow at room temperature, however the opportunistic thermophilic fungi such as *Aspergillus sp*, can enter the respiratory tract and cause infection (Portnoy et al. 2005). If the airborne fungal spores are inhaled down to the bronchia and alveoli, they will be lysed and the human body will be exposed to primary and secondary metabolites (Fischer and Dott 2003).

An investigation by Jarvis and Morey in 2001, on a contaminated 11-story structure located in the southeast United States, found the building to have moisture problems mainly throughout the building's walls. The building's operator located the moisture problem within the gypsum board of the envelope walls directly under vinyl

المنارة للاستشارات
www.manaraa.com

wall coverings. Before the end of the first year, the building operator was removing the vinyl wall covering from some walls and applying paint to the underlying paper fiber gypsum board in an attempt to lower the moisture. Before the end of the year, visible fungal colonization appeared throughout the building. The investigation discovered that many occupants had experienced hay fever and asthma conditions.

Singh and Yu (2010) suggests the best strategy for remediating mold problems depends on certain circumstances, including the severity of the problem, the number of affected persons, economic concerns, and whether or not the building can be vacated for a time period to undertake remediation work. Furthermore there are ways to reduce indoor air pollutants either by source control, which is the most effective method thereby preventing or limiting mold development, and the other is to remove the sources.

Wall Construction

Wood, an important natural resource throughout the world, was used by early American settlers, which helped allow the transition from huts of logs to sawed lumber (Parker et al. 1958). The primary sources of wood are usually located in less dense populations in the United States, Canada, Australia, New Zealand, Russia, and certain Latin American countries. The process of transitioning wood from logs to timber usually involves transporting the logs from the forest to the mill, debarking of logs, and sawing the debarked logs into lumber. The woods most commonly used for exterior walls include spruce, hemlock, southern yellow pine (Pinus taeda), and larch for wood studs based on durability and strength. In addition to wood studs, wood panels are an important part of wood frame construction. An example commonly used is oriented strandboard (OSB), and plywood which are for structural sheathing. Plywood panels are composed of wood

المنارة للاستشارات
www.manaraa.com

veneer, produced by a machine that holds a debarked log at two ends, rotates the log against a stationary knife blade resulting in slicing of a continuous veneer about 1/8 in. thick. The veneer ply's are glued under heat and pressure (Mehta et al. 2008) in alternating grain direction. However due to the recent desire to use resources more efficiently, plywood panels have changed to OSB. OSB is called oriented strandboard because alternate layers of wood strands are oriented at right angles. OSB panels are made by gluing several layers of wood strands under heat and pressure (Mehta et al. 2008). Components that make up the exterior wall include exterior siding such as brick, wood, or vinyl siding. Vinyl siding is composed of polyvinyl chloride (PVC), thermoplastic polyolefin (TPO), or polypropylene. Due to its properties, vinyl siding makes for a more durable, less expensive, and water resistant material (Mehta et al. 2008).

The interior walls are usually nonstructural but serve as dividers and contain mechanical and electrical components. However, the effect of insulation within the wall is very important in reducing energy. Insulating materials that are flexible are commonly used in stud walls and may be in the form of blankets, batts, or loose-fill insulation. Due to an energy crisis, about 20-30 years ago, helped allow the development of newer homes with air-tight structures in order to conserve energy. It has been suggested by Lstiburek (2007) that not only new construction methods are energy efficient and affordable, but they are prone to ageing and biodeterioration, which can cause structural damage. The main change has been a reduction in drying potential of the enclosed wall spaces and adding thermal resistance compared to the older types of construction (Lstiburek 2009). With the addition of thermal resistance, the newer types of wall structures are unable to

www.manaraa.com

dry completely. The increased ability of moisture to impact the wood product is due to the transition to plastic vapor barriers, vinyl wall coverings, and foil faced fiberglass batt insulation, which allows accumulated water to exceed the moisture storage capacity of the material (Lstiburek 2009). Lstiburek (2007) also states that refined wood products make the mold nutrients more accessible and susceptible to mold growth. Unfortunately, water penetrating through the newer types of building material could lead to water leakage into the building cavities and cause problems for homeowners.

Figure 1.1 shows the building materials and design common today in wall construction. The components include the gypsum wallboard, solid wood stud, batt insulation, exterior sheathing, house wrap, and exterior siding. The interior wall component, the gypsum wallboard, was first used in about 1915 primarily for remodeling purposes. Gypsum wallboard gradually evolved after World War II resulting in an improved surface appearance and construction (Simmons 2001). Gypsum is a rocklike mineral, and the board product is commonly referred to as drywall, wallboard, and plasterboard. It has a low moisture content. Gypsum wallboard (Figure 1.1A) consists of a gray paper liner on the back of the gypsum core with calendared manila paper on the front, providing a smooth, even finish for decorating (Mehta et al. 2008). According to Simmons (2001), the use of solid wood studs originated around the middle of the nineteenth century in the United States, and changed the direction of the housing industry. This product allowed for a rapid production of houses and other wood frame structures to be constructed. Prior to the use of wood studs, frame buildings were built of heavy timbers, mortised, tendoned together, and pinned with hardwood dowels. A current wood stud (Figure 1.1B) is a solid 2x4 or 2x6 untreated southern yellow pine board and is

المنارة للاستشارات
www.manaraa.com

spaced 16 or 24 inches to accommodate wall sheathing and finish materials, as well as provide the structural framework for the wall.

المنارة للاستشارات
www.manaraa.com

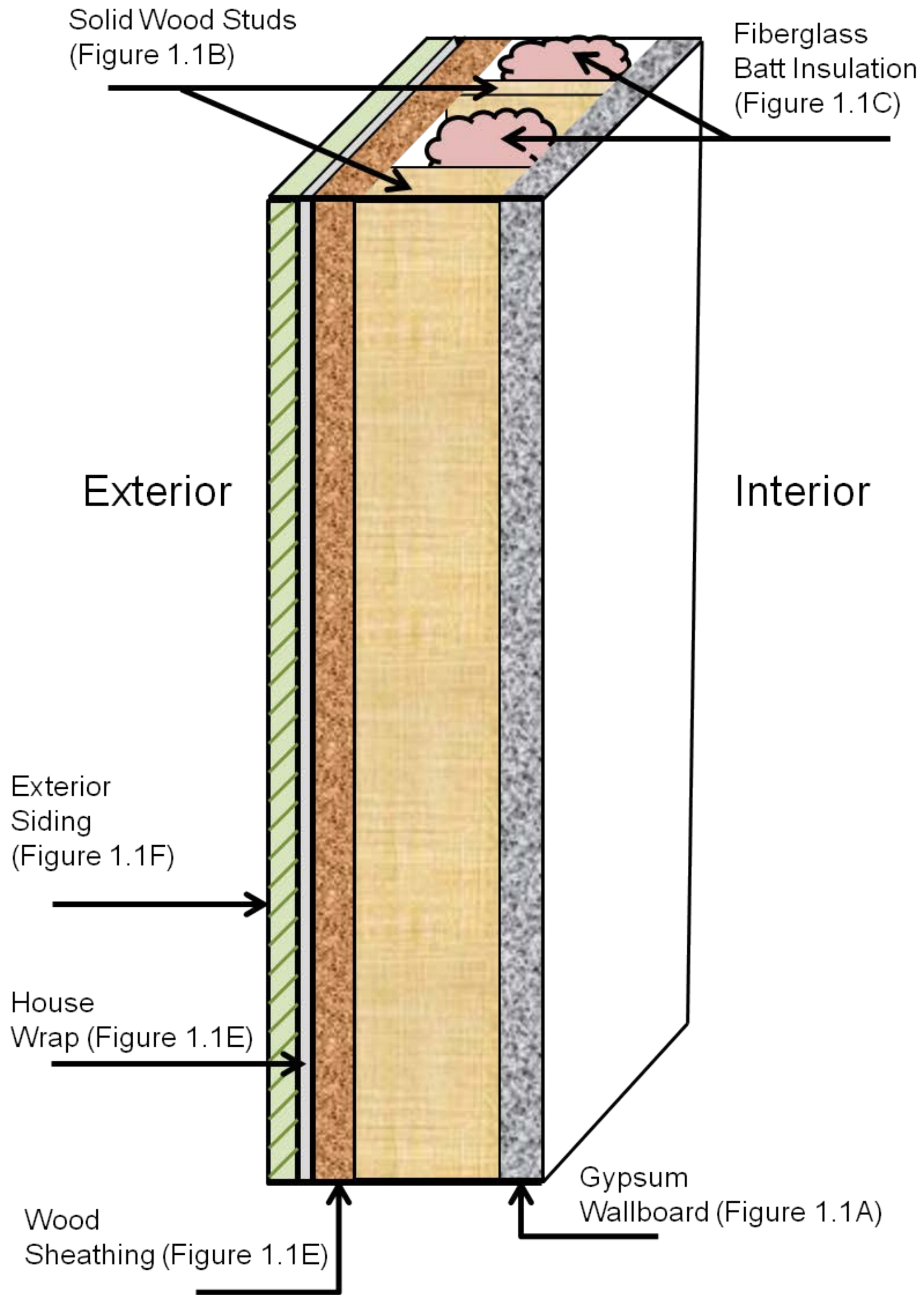

Figure 1.1 Diagram of building materials used for wall construction.

المنارة للاستشارات
www.manaraa.com

Due to economic and comfort considerations, modern buildings are being built tighter and kept warmer in winter and cooler in summer as compared to buildings built 50 or more years ago (Simmons 2001). In some climates, using structural or finish materials with high thermal capabilities is sufficient in controlling heat gain or loss through a building's envelope allowing for a healthy and comfortable indoor environment. Wall insulation is most commonly made of fiberglass, but may also be made from mineral wool, plastic fibers, or natural fibers. It comes in rolls (or batts) that are typically sized to fit standard construction spacing. Batt wall insulation (Figure 1.1C) is placed between wood studs or stapled to wood stud (Mehta et al. 2008). Batt insulation may come with a facing which is typically a kraft paper-faced product, foil-kraft paper or vinyl. This facing functions as a vapor retarder. Some sites recommend not using faced batt insulation in hot, humid environments due to the buildup of moisture in the walls. Batt insulation also comes in different R-values, which is a measure of the insulation capacity. For exterior walls, an R-value of R-13 to R-21 is commonly used. Exterior grade sheathing (Figure 1.1D) is applied over wood-framed construction and may include plywood, OSB, or non-wood material. The purpose for sheathing is to structurally frame the building, reduce heat transmission, resist moisture infiltration, and function as a nailing base for exterior finish materials (Simmons 2001). The difference between OSB and plywood panels is that OSB provides higher shear strength than plywood because of the absence of core voids. However, the author further states that plywood panels can be stained or painted whereas OSB panels are intended only for structural applications. Additionally, OSB tends to be less expensive than plywood, thus is most commonly used today. House wrap was initially discovered in 1955, and gradually evolved into DuPont™ Tyvek®

المنارة للاستشارات
www.manaraa.com

CommercialWrap®, which allows the protection against air and water infiltration while allowing moisture vapor to escape the wall cavity. House wrap is commonly applied on the exterior side of the wall sheathing (Figure 1.1E). Exterior siding can include brick, wood siding, stucco, or vinyl siding (Figure 1.1F). Vinyl siding is made from polyvinyl chloride (PVC) compound with additives such as lubricants, stabilizers, impact modifiers, ultraviolet inhibitors, and color (Simmons 2001). Installing vinyl or wood siding requires the overlapping of long strips either horizontal or vertical and nailed with corrosion-resistant fasteners to the studs or wall sheathing (Mehta et al. 2008).

Identification of Mold

The construction of a building can lead to mold contamination depending on the location and region, which includes various types of environments found within the United States (Dillion et al. 1999). In addition to the differences in climate, the displacement of the vapor barrier in the wall cavity or lack of ventilation within the building can lead to sources of mold development. Anderson et al. (2011) suggests that detection and species identification of all fungi present in a moldy building is essential for resolving the cause and effect of building-related illness. Furthermore, the author states that the common types of identification of mold vary depending on the type of testing needed to identify the mold species. The main reasons for sample collection when fungal contamination is suspected is to detect, quantify, and identify fungi that might be present because airborne particles could include microorganisms (both fungi and bacteria), pollen, fragments from animals, and manmade particulates such as soot (Portnoy et al. 2004). Furthermore the authors states that a typical sampling protocol should include measurement of temperature and relative humidity in all major areas of

المنارة للاستشارات
www.manaraa.com

the building. The methods used for identifying fungi involve a visual inspection to identify sources of fungal contamination by focusing on locations near moisture sources. Moisture sources can include condensation and intrusion. Condensation occurs when air comes into contact with a surface that has a temperature of less than the dew point of the air, which is the temperature at which air has 100% humidity. Examples of condensation areas can typically be found around windows. The other moisture source, intrusion, occurs when water enters a building from external sources, such as leaks, floods, or groundwater penetration (Portnoy et al. 2004).

When a building becomes compromised, the traditional methods of identification involve culturing the mold contaminant and using a microscope with taxonomic keys to distinguish the morphological characteristics of the fungi. Reliable use of a taxonomic key, however, requires expertise due to the similar appearances or very minor differences among some species of fungi. Anderson et al. (2011) studied the qualitative and quantitative diversity of fungi growing on 5300 surface samples taken by cultivation contact plates from building materials, and reported that contact plates allowed direct genus identification of common indoor molds. It has been found, however, that all sampling methods based on cultivation detect only viable fungal spores which can be allergenic or potentially harmful to individuals. In addition, some mold species grow very rapidly, while others grow more slowly, and this may result in one species over-running others on the culture plates, thus some species may be missed.

Other methods such as surface or air sampling to test for airborne spores help detect mold growth that may be hidden. The most common surface sampling technique is tape-lifting sampling in which tape is placed onto the discoloration area, then stained and

المنارة للاستشارات
www.manaraa.com

placed sticky side down on a microscope slide. This method allows a rapid detection for the presence of fungal spores, can be useful in identifying fungi genera, and provides a semi quantitative estimation of the amount of each type of fungi. In addition to the tape-lift method, viable spore sampling of a discolored area is more time consuming because it depends on fungal growth since some species of fungi grow at different rates. The typical sampling method is to use a device such as a cotton swab or plastic loop, with a stabilization media for transport. The advantage of a fungal culture is that it can be used to identify specific species, whereas the tape-lift method is accurate only to the genus level. However the downside is that the method of culturing fungi takes longer, allows for rapidly growing species to dominate others, and some molds such as *Stachybotrys* species grow poorly on most media (Portnoy et al. 2004).

In addition to surface sampling, air sampling measures spore concentration in air along airflow pathways using different devices to measure the concentration of spores at a single time and location. The advantages of air sampling are the identification of species is possible, separation of organisms by size, and spore enumeration (Portnoy et al. 2004). Meklin et al. (2007) states that some limitations of air sampling occur during testing which results in decreased growth since most species don't grow well on the same media or grow at the same rate as others, failure to identify mold as a specific species, and the possibility of overgrowth occurring on culture plates. Anderson et al. (2011) studied the detection of fungi by air sampling, and reported that some types of fungi that produce large quantities of small, dry spores, such as *Aspergillus spp*., *Cladosporium spp.,* and *Penicillium spp*. are easily detected, however the fungal species that produce small amounts of spores, large spores, or spores in slime, such as *Acremonium spp*.,

المنارة للاستشارات
www.manaraa.com

Trichoderma spp., *Stachybotrys spp*. and *Chaetomium spp*, may not be as easily detected during air sampling. Gorny et al. (2001) studied the release of *Aspergillus versicolor*, *Cladosporium cladosporioides*, and *Penicillium melinni* spores within an aerosolization chamber and concluded that depending on the thickness of conidiophores, length of spore chains, and shape of spores, typical indoor air currents can release up to 200 spores cm^{-2} from ceiling tile surfaces.

Meklin et al. (2004) states that although the use of common methods such as visual inspection and traditional culture methods is considered to be the best sampling methods for identifying building mold problems, performing these procedures can require considerable time, labor, and expense. Furthermore the author suggests that using a DNA-based method for identifying and quantifying mold species can be inexpensive, more rapid, and a standardized method for mold analysis that could lead to reducing costs. The first report of the process of polymerase chain reaction (PCR) was conducted by Mullis et al. (1986) who suggested that the discovery of specific restriction endonucleases led to the exploration of an alternative method for the synthesis of specific DNA sequences. This method involves repetitive cycles of denatuaration, hybridization, and polymerase extension, which increases the concentration of DNA exponentially. However, molecular procedures such as cloning and sequencing of genomic DNA are needed to identify the organism. Landeweert et al. (2003) state that molecular identification techniques based on rapid genomic DNA extraction provide a novel approach to examine fungal communities. The fungal DNA can be amplified using PCR with a pair of primers which allows specific amplification of the fungal ribosomal DNA. Furthermore, the construction of fungal clonal libraries by 18S rDNA and internal

المنارة للاستشارات
www.manaraa.com

transcribed spacer (ITS) regions enables the identification of fungal species. It has been discovered that some mold species can be detected better with PCR than with traditional methods such as microscopy and culture. Chew et al. (2006) concluded that PCR can not only detect dead fungi but also those that compete poorly. According to Bellanger et al. (2009), PCR has many advantages compared to other methods such as no significant expertise in mycology is required for species identification, dead spores that possess allergenic properties can be investigated, and the detection and quantification can be standardized. Their work indicated that the use of real-time PCR was able to detect the toxic mold *Stachybotrys chartarum*, in surface samples from 21 moisture-damaged homes, whereas it was isolated only once by culture method.

In addition to identification, methods allowing quantification of mold on building materials are important in evaluating mold damage in buildings and the quality of remediation efficacy (Reeslev et al. 2003). The US Environmental Protection Agency has established the Environmental Relative Moldiness Index (ERMI), which each indoor mold species is grouped into Group 1 and Group 2 molds. The Group 2 molds are known to be common in moist homes and in low concentrations, which have little impact on the health of residents. However, it was found that Group 1 molds significantly impacted the health of residents of homes that have been affected by water intrusion because of poor construction or leaking pipes (Sobeck 2007). Before the development of molecular based methods for quantification, the traditional methods were based on the enumeration of cells captured on a sticky surface and counted under a microscope or by culturing molds from the sample on various media. However, most mold colonies cultured can look similar on some media but not the same on other types. Furthermore cells could form

المنارة للاستشارات
www.manaraa.com

clumps which will produce a single colony and result in an inaccurate estimation of mold concentration (Vesper 2011). Another factor that could impact the estimation of mold present within building is the presence of fungal fragments. Fungal fragments have been overlooked when assessing exposures in moldy buildings. According to Reponen et al. (2007), the determination of fungal fragments is very important since some fragments have the ability to live longer in the air as compared to larger spores and can penetrate deeply into the lungs when inhaled. However as the author suggests, the quantification of fungal fragments has been difficult due to the lack of suitable field-compatible sampling and analysis methods.

Other methods that measure quantitative loads of fungi species such as β-glucan and ergosterol can be used to estimate fungal biomass on building materials. However, the determination of the ergosterol content is rarely used in the determination of mold because ergosterol is not produced by all fungi and concentrations vary between the same species depending on the physiology of the fungus (Chiocchio et al 2011).

The development of real-time PCR in the early 1980s provided a molecular method to amplify specific regions of genomic DNA unique to the species of mold (Vesper 2011). According to Sobeck (2007), due to the breakthrough of using quantitative PCR for mold sampling, the technology can be used accurately to detect not only which species of mold are present but also how much of a species is contaminating the indoor environment. The advantage of real-time PCR is its ability to measure the amplified PCR product at each PCR cycle. Therefore this allows the exponential phase of the reaction to be monitored and allows an accurate quantification of DNA template (Lievens et al. 2006). According to this author, the use of real-time PCR, allowed for

المنارة للاستشارات
www.manaraa.com

control measures to be determined more rapidly and accurately than the conventional methods of isolation and quantification. Wong and Medrano (2005) states that the major disadvantage to real-time PCR is that it requires expensive equipment and reagents.

Mold, Moisture and Building Materials

According to Ashley et al. (2009), flooding caused by Hurricane Katrina in Louisiana, resulted in contaminated sediments left behind from Lake Pontchartrain being deposited inside homes and businesses. The authors further state that after the floodwaters had drained, first responders, returning residents, and recovery workers were exposed to pollutants by dermal contact with sediments and inhalation of contaminated mold aerosol. Experiments have been conducted to determine the types of fungi species within water-damaged homes using different identification methods. Fungal species found by Anderson et al. (2011) within water-damaged homes via air sampling using multivariate statistics determined that there was an association between fungi and material components. These workers reported that *Penicillium chrysogenum* and *Aspergillus versicolor* were the most common fungi species found in water-damaged buildings. Finally, their results showed an association between *Acremonium spp., Penicillium chrysogenum, Stachybotrys spp.,* and *Ulocladium spp.* on gypsum, between *Arthrinium phaeospermum, Aureobasidium pullulans, Cladosporium herbarum, Trichoderma spp.*, on different types of wood and plywood, and *Aspergillus spp., Chaetomium spp., Mucor spp.* on concrete and other floor materials. In other studies, Chew et al. (2006) used PCR to detect 23 mold species in air samples before, during, and after renovation of three New Orleans homes. The common molds were *Aspergillus, Penicillium,* and *Cladosporium*, but *Stachybotrys* was only found 40 percent of the time.

المنارة للاستشارات
www.manaraa.com

Bloom et al. (2009) was able to detect *Stachybotrys* in all dust samples but one using PCR, but did not identify this species in a single cultured sample.

Overview of Research

The overall objective of this investigation was to identify and quantify mold species within a small flood unit on building materials provided by Tuskegee University. More specifically, the aims of this study are to (1) evaluate the location of each type of mold fungi on building products located both above and below the water line including batt insulation, gypsum wallboard, wood stud, plywood sheathing, vinyl siding, and house wrap, (2) determine the identity of each mold fungi using cloning and sequencing procedures, (3) determine the quantity of select mold fungi using real-time PCR.

المنارة للاستشارات

www.manaraa.com

CHAPTER II

MATERIALS AND METHODS

Background

A small building was built at Tuskegee University and exposed to flood conditions similar to flooding that occurred during Hurricane Katrina in New Orleans, LA in 2005. This Flood Unit was built to residential code and in May 2010 was flooded using pond water to a depth of two feet (Figure 2.1). The Unit remained flooded for three weeks and then water was drained. The Flood Unit was kept closed for an additional three weeks. The Flood Unit was then opened (Figure 2.2) and different building material sections removed for mold analysis.

المنارة للاستشارات
www.manaraa.com

Figure 1.2 Flood Unit testing site at Tuskegee University (Tuskegee, AL). Photograph Courtesy of Tuskegee University.

المنارة للاستشارات
www.manaraa.com

Figure 1.3 Flood Unit opened after three weeks showing mold development. Photograph Courtesy of Tuskegee University.

The following wall samples used in this study were supplied by Dr. Heshmat Aglan of Tuskegee University (Tuskegee, AL): Fiberglass Batt Insulation, Gypsum Board, Solid Wood Stud, Vinyl Siding, Wood Sheathing (Plywood), and House Wrap.

The first sets of samples were received June 2010 and are termed 'Wet Samples.' These samples consisted of batt insulation, four samples of gypsum board numbered 1 through 4, plywood sheathing associated with gypsum board #4, and wood stud sample #7 associated with house wrap and vinyl siding. Each sample, when possible, was separated into exposure above the water line and below the water line. These sections were further dissected, when possible, into locations with term Front referring to the side of the material that was facing into the house, Rear referring to the side of the material

المنارة للاستشارات
www.manaraa.com

that was facing into the wall cavity and Middle referring to the portion of the product between Front and Back. Figure 2.3 shows the different sections of gypsum wallboard.

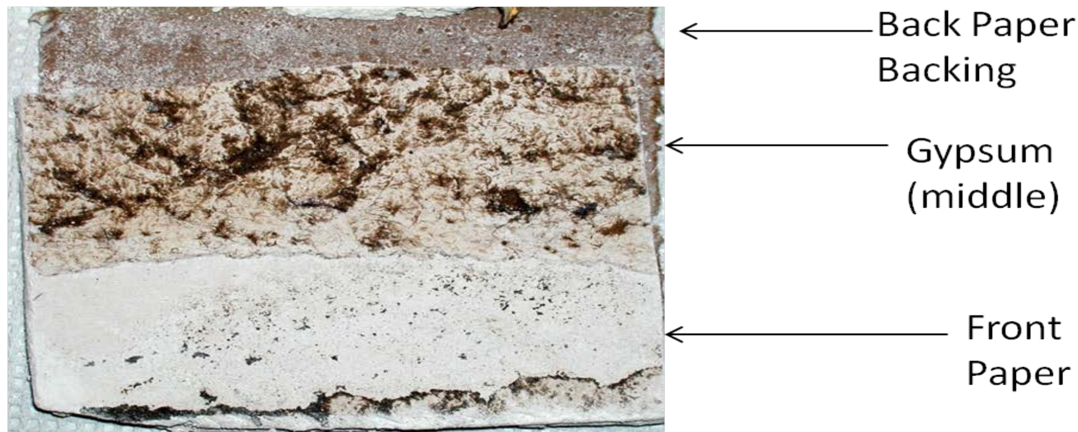

Figure 1.4 The different sections of gypsum wallboard

Three replicates were taken from each of these locations for each material and sample codes are listed in Table 2.1.

Table 2.1 Wet Building Materials Obtained from Flood Unit.

Sample	Sample Code	Above or Below Water Line	Location
Batt Insulation #5&6	B1AP1	Above	Paper on Insulation
	B1AP2	Above	Paper on Insulation
	B1AP3	Above	Paper on Insulation
	B1BP1	Below	Paper on Insulation
	B1BP2	Below	Paper on Insulation
	B1BP3	Below	Paper on Insulation
	B1AN1	Above	Insulation behind paper
	B1AN2	Above	Insulation behind paper
	B1AN3	Above	Insulation behind paper

المنارة للاستشارات
www.manaraa.com

Table 2.1 (continued)

	B1BN1	Below	Insulation behind paper
	B1BN2	Below	Insulation behind paper
	B1BN3	Below	Insulation behind paper
	B1AF1	Above	Insulation at back Far from paper
	B1AF2	Above	Insulation at back Far from paper
	B1AF3	Above	Insulation at back Far from paper
	B1BF1	Below	Insulation at back Far from paper
Gypsum Board#3	G3AS1	Above	Paper Surface Front
	G3AS2	Above	Paper Surface Front
	G3AS3	Above	Paper Surface Front
	G3BS1	Below	Paper Surface Front
	G3BS2	Below	Paper Surface Front
	G3BS3	Below	Paper Surface Front
	G3AR1	Above	Paper Surface Rear
	G3AR2	Above	Paper Surface Rear
	G3AR3	Above	Paper Surface Rear
	G3BR1	Below	Gypsum Surface Rear
	G3BR2	Below	Gypsum Surface Rear
	G3BR3	Below	Gypsum Surface Rear
	G3AM1	Above	Gypsum Middle of Board
	G3AM2	Above	Gypsum Middle of Board
	G3AM3	Above	Gypsum Middle of Board
	G3BM1	Below	Gypsum Middle of Board
	G3BM2	Below	Gypsum Middle of Board
	G3BM3	Below	Gypsum Middle of Board
Gypsum Board#4	G4AS1	Above	Paper Surface Front
	G4AS2	Above	Paper Surface Front
	G4AS3	Above	Paper Surface Front
	G4BS1	Below	Paper Surface Front
	G4BS2	Below	Paper Surface Front
	G4BS3	Below	Paper Surface Front
	G4AR1	Above	Paper Surface Rear
	G4AR2	Above	Paper Surface Rear
	G4AR3	Above	Paper Surface Rear
	G4BR1	Below	Paper Surface Rear
	G4BR2	Below	Paper Surface Rear
	G4BR3	Below	Paper Surface Rear
	G4AM1	Above	Gypsum Middle of Board
	G4AM2	Above	Gypsum Middle of Board
	G4AM3	Above	Gypsum Middle of Board

المنارة للاستشارات
www.manaraa.com

Table 2.1 (continued)

	G4BM1	Below	Gypsum Middle of Board
	G4BM2	Below	Gypsum Middle of Board
	G4BM3	Below	Gypsum Middle of Board
Gypsum Board#2	G2TS1		Paper Surface Front/ Top of Board
	G2TS2		Paper Surface Front/ Top of Board
	G2TS3		Paper Surface Front/ Top of Board
	G2BS1		Paper Surface Front/ Bottom of Board
	G2BS2		Paper Surface Front/ Bottom of Board
	G2BS3		Paper Surface Front/ Bottom of Board
	G2TR1		Paper Surface Rear/ Top of Board
	G2TR2		Paper Surface Rear/ Top of Board
	G2TR3		Paper Surface Rear/ Top of Board
	G2BR1		Paper Surface Rear/ Bottom of Board
	G2BR3		Paper Surface Rear/ Bottom of Board
	G2TM1		Gypsum Middle of Board/ Top of Board
	G2TM2		Gypsum Middle of Board/ Top of Board
	G2TM3		Gypsum Middle of Board/ Top of Board
	G2BM1		Gypsum Middle of Board/ Bottom of Board
	G2BM2		Gypsum Middle of Board/ Bottom of Board
	G2BM3		Gypsum Middle of Board/ Bottom of Board
Gypsum Board#1	G1AS1	Above	Paper Surface Front
	G1AS2	Above	Paper Surface Front
	G1AS3	Above	Paper Surface Front
	G1BS1	Below	Paper Surface Front
	G1BS2	Below	Paper Surface Front
	G1BS3	Below	Paper Surface Front
	G1AR1	Above	Paper Surface Rear
	G1AR2	Above	Paper Surface Rear
	G1AR3	Above	Paper Surface Rear
	G1BR1	Below	Paper Surface Rear
	G1BR2	Below	Paper Surface Rear

المنارة للاستشارات
www.manaraa.com

Table 2.1 (continued)

	G1BR3	Below	Paper Surface Rear
	G1AM1	Above	Gypsum Middle of Board
	G1AM2	Above	Gypsum Middle of Board
	G1AM3	Above	Gypsum Middle of Board
	G1BM1	Below	Gypsum Middle of Board
	G1BM2	Below	Gypsum Middle of Board
	G1BM3	Below	Gypsum Middle of Board
Wood Sheathing#7	W7AS1	Above	Surface Front
	W7AS2	Above	Surface Front
	W7AS3	Above	Surface Front
	W7BS1	Below	Surface Front
	W7BS2	Below	Surface Front
	W7BS3	Below	Surface Front
	W7AR1	Above	Surface Rear
	W7AR2	Above	Surface Rear
	W7AR3	Above	Surface Rear
	W7BR1	Below	Surface Rear
	W7BR2	Below	Surface Rear
	W7BR3	Below	Surface Rear
House Wrap#7	I7A1	Above	
	I7A2	Above	
	I7A3	Above	
	I7B1	Below	
	I7B2	Below	
	I7B3	Below	
Vinyl Siding#7	V7A1	Above	
	V7A2	Above	
	V7A3	Above	
	V7B1	Below	
	V7B2	Below	
	V7B3	Below	
Wood Stud#4	W4AS1	Above	Surface Front
	W4AS2	Above	Surface Front
	W4AS3	Above	Surface Front
	W4BS1	Below	Surface Front
	W4BS2	Below	Surface Front
	W4BS3	Below	Surface Front

المنارة للاستشارات
www.manaraa.com

Table 2.1 (continued)

	W4AR1	Above	Surface Rear
	W4AR2	Above	Surface Rear
	W4AR3	Above	Surface Rear
	W4BR1	Below	Surface Rear
	W4BR2	Below	Surface Rear
	W4BR3	Below	Surface Rear

Additional wall materials were allowed to dry within the Flood Unit for seven months. These samples termed 'Dry Samples' were then removed on February 2011 and sent for analysis. They include two gypsum board samples and two solid wood stud samples. These samples were separated as described for the wet samples and sample codes are listed in Table 2.2.

Table 2.2 Dry Building Materials Obtained from Flood Unit.

Sample	Sample Code	Above or Below Water Line	Location
Wood Stud#1	S1AF1	Above	Front w/ writing
	S1AF2	Above	Front w/ writing
	S1AF3	Above	Front w/ writing
	S1AM1	Above	Middle
	S1AM2	Above	Middle
	S1AM3	Above	Middle
	S1AR1	Above	Rear
	S1AR2	Above	Rear
	S1AR3	Above	Rear
	S2BF1	Below	Front w/ writing
Wood Stud#2	S2BF2	Below	Front w/ writing
	S2BF3	Below	Front w/ writing
	S2BM1	Below	Middle

للاستشارات المنارة
www.manaraa.com

Table 2.2 (continued)

	S2BM2	Below	Middle
	S2BM3	Below	Middle
	S2BR1	Below	Rear
	S2BR2	Below	Rear
	S2BR3	Below	Rear
Gypsum #3	G3AF1	Above	Front white paper
	G3AF2	Above	Front white paper
	G3AF3	Above	Front white paper
	G3AM1	Above	Middle
	G3AM2	Above	Middle
	G3AM3	Above	Middle
	G3AR2	Above	Rear brown paper
	G3AR3	Above	Rear brown paper
Gypsum #4	G4BF1	Below	Front white paper
	G4BF2	Below	Front white paper
	G4BF3	Below	Front white paper
	G4BM1	Below	Middle
	G4BM2	Below	Middle
	G4BM3	Below	Middle
	G4BR1	Below	Rear brown paper
	G4BR2	Below	Rear brown paper
	G4BR3	Below	Rear brown paper

Preparation of Building Materials

Each material sample as listed in Table 2.1 and 2.2 were reduced to smaller pieces using sterile scissors, knives, or sterile scrapers for wood stud. A portion of these pieces were processed for DNA analysis, a portion of some samples were plated for fungal culture isolations and the rest was frozen at -20 degree C.

DNA Extraction

DNA Extraction was conducted by using the Nucleospin Plant II Kit (Machery Nagel, Duren, Germany). Each type of sample and mycelia pure cultures were weighed

المنارة للاستشارات
www.manaraa.com

and placed into 2mL capped tubes with two sterile 5mm beads and 1mL 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) including 20µl Rnase A for the wood material samples and 17µl of RNase A for the mycelia pure culture samples with 17µl Proteinase K to each sample tube. Samples and mycelia pure cultures were placed onto a Biospec Mini Beadbeater bead mill (Bartlesville, OK) for three minutes. After two cycles for three minutes at maximum speed on the beader mill, the samples were placed in a water bath at 65º C for two hours. After the water bath, a Nucleospin filter column was placed into a new collection tube to transfer the lysate and centrifuged for two minutes at 11,000rpm. After centrifugation, all supernatant was transferred into a new 1.5 mL micro centrifuge tube and 900µl of buffer PC was mixed thoroughly. After mixing, a Nucleospin plant column was placed into a new collection tube, adding 700µl of the solution to the column. After adding the solution, the column was centrifuged for one minute at 11,000 rpm. After allowing all lysate to be passed through the column, 400µl of buffer PW1 was added to the Nucleospin plant column and centrifuged at 11,000rpm for one minute. After discarding the flow through, 700µl of buffer PW2 was added to the column and centrifuged for 11,000rpm for one minute. After one minute, the flow through was discarded and 200µl of buffer PW2 was added to the column and centrifuged for 11,000rpm for two minutes to dry the membrane. After drying, the Nucelospin plant column was placed into a new 1.5ml micro centrifuge tube and 50µl of 70°C preheated elution buffer PE was pippetted directly onto the membrane. After 5 minutes at room temperature and centrifugation at 11,000rpm an additional 30µl of

المنارة للاستشارات
www.manaraa.com

preheated buffer PE was placed onto the membrane for five minutes and centrifuged at 11,000rpm for one minute for a total of 80µl of PE.

Polymerase Chain Reaction (PCR) Amplification

After DNA extraction, each sample and pure cultures were selected for PCR amplification using an Eppendorff Thermocycler (Hamburg, Germany). The eluted DNA in addition to the Ultra pure water (Sigma) was added to a Master Mix consisting of 25mM MgCl (Fisher), 10mM Buffer (Fisher), 5 units/µl Taq DNA polymerase enzyme, and 10mM forward NF (5`GTAGTCATATGCTTGTCTC-3`), and 10mM reverse NR (5`CTTCCGTCAATTCCTTTAAG-3`) primer. The solution was added in 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 was added with the Taq polymerase into each PCR tube and placed on 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 DNA. Once the DNA was amplified, the product was analyzed by using gel electrophoresis.

Gel Electrophoresis was accomplished by making a 2% agarose gel following specified procedures for either a 60mL or 100mL gel system using Sodium Boric Acid (SBA) buffer. The solution was microwaved for ~ 1 minute until boiling. After boiling, 3.5µl of either GelStar or GelRed was added to 60mL or 7µl to the 100mL gel solution. The solution was poured into a gel box and covered with box tray to keep out dust and the gel was set for one hour to solidify. After an hour, running buffer was added to cover the gel. 10µl of DNA was added to 2µl of blue dye and pipetting up and down until solution was mixed. After loading the gel with blue dye and DNA, the gel was run for ~1

المنارة للاستشارات
www.manaraa.com

½ hours at 90 volts on the EC4000P voltage 90 machine to analyze if successful amplification had occurred.

Direct Purification of PCR product

The samples which were selected for cloning based on expression of 18S from the gel electrophoresis went through a direct purification to remove excess PCR reagents. The protocol for purification involved mixing 40µl of PCR product with 80µl of Buffer NT. To bind the DNA, a Nucleospin Extract II (Machery-Nagel) column was placed inside a 2 mL collecting tube with the sample loaded. After loading, the sample was centrifuged for one minute at 11,000 rpm. After centrifugation, the flow-through was discarded and the Nucleospin Extract column was placed into the collecting tube. After placing the column into the collecting tube, 600µl of buffer NT3 was added to wash silica membrane and centrifuged for one minute at 11,000rpm. After one minute, the flow-through was discarded and the NucleoSpin Extract II was placed into the collecting tube. To dry the silica membrane, the NucleoSpin Extract II was centrifuged for an additional 2 minutes at 11,000rpm to remove remaining buffer NT3. To elute the DNA, the NucleoSpin Extract II column was placed into a clean 1.5mL microcentrifuge tube and 35µl of pre-warmed elution buffer NE was added. After five minutes at room temperature, the sample was centrifuged for one minute at 11,000rpm to elute the DNA.

المنارة للاستشارات
www.manaraa.com

Media for Cloning

1. Ampicillin Stock:

   Ampicillin Stock was produced by weighing 0.1 g ampicillin (Sigma) into tube and adding 1mL distilled water. The mixture was mixed by using a vortex to dissolve into solution. Once dissolved, the solution was stored in refrigerator at 4ºC

2. Luria Plates with 100µg/ml ampicillin:

   Luria Plates was prepared by weighing 8 grams of Luria- Bertani Agar obtained from Fisher Scientific (Fairlawn, NJ). A 500 mL glass flask was used to mix the 200mL of water and agar by using a stir bar. After mixing, the solution was autoclaved, the solution cooled to 55 degree C and 200µl of ampicillin stock was added. After ampicillin was added, the agar was dispensed into the petri dishes at a setting specified for 20mL/plate.

3. Luria Broth with 50 µg/ml ampicillin:

   Luria Broth was prepared by weighing 2.5 grams of Luria-Bertani Broth purchased from Fisher Scientific (Fairlawn, NJ). A 250mL glass flask was used to mix the 100mL of water and broth by using a stir bar. After mixing, the solution was autoclaved and after cooling, 50µl of ampicillin stock was added. After ampicillin was added, the broth was dispensed into sterile plastic tubes for 2mL at a setting specified.

Cloning Procedure

The selected samples from each type of sample set went through the process of cloning using the Invitrogen TOPO TA Cloning Kit (Carlsbad, CA). To insert the foreign

المنارة للاستشارات
www.manaraa.com

DNA through ligation with vector DNA, 1µl or 2µl DNA fragment was mixed with 3µl or 2µl of water plus 1µl salt solution and 1µl pCR 4-TOPO vector. The solution was placed into a 500µl tube on ice for five minutes of incubation at room temperature. For transformation to occur, 2µl of the TOPO Cloning reaction was injected into a vial of competent One Shot TOP10 *Escherichia coli* and incubated on ice for thirty minutes. After incubation, the cells were heat shocked for thirty seconds by placing into a water bath at 42 degrees C before being placed on ice for two minutes. After two minutes on ice, 250µl of SOC was added before shaking horizontally (200rpm) for one hour at 37 degree C. The samples were then plated using spread plate method using 50µl or 100µl with 20µl of SOC on prewarmed selective Luria-Bertani (LB) agar containing ampicillin. Plates were incubated at 37 degrees C for twenty four hours and accessed for colony formation. Ten colonies were picked off the plate that showed good transformation and inoculated into LB broth and placed on a shaker at 200rpm at 37 ºC. After twenty four hours the samples labeled A-J, were extracted using the Quick Plasmid Miniprep Kit (Carlsbad, CA) for plasmid DNA.

Plasmid Extraction

Plasmid extraction was achieved by pipetting 1mL of the overnight culture into 1.5mL tubes. The tubes were placed into a centrifuge for 7,000rpm for 5 minutes. After centrifugation, the supernatant was removed and the cell pellets remained. The cell pellets were resuspended in 250µl resuspension buffer (R3) containing Rnase A and vortexed to break the pellet. Then the 250µl of lysis buffer (L7) was added and inverted five times to mix the solution. After the lysis buffer and five minutes of incubation at room temperature, 350µl precipitation buffer (N4) was added to each 1.5mL tube and

المنارة للاستشارات
www.manaraa.com

mixed until the solution was homogeneous. The tubes were placed into the centrifuge for 10,000rpm for 10 minutes and pippetted into columns. Buffers W9 and W10 was added to wash each column twice before eluting the plasmid DNA with 75μl of preheated TE buffer. The purified plasmid DNA was stored in -20 degree C before cycle sequencing and analysis.

Enzyme Digest

Analysis for the insert of the foreign DNA was conducted using an enzyme digest. The Master Mix was prepared by adding 2μl of Promega 10x dilution Buffer solution (Madison, WI), 13.8μl of Ultra pure water (Sigma), 0.2μl of Promega 100x dilution Bovine Serum Albumin Acetylated (Madison, WI), and 0.5μl of Promega Enzyme EcorI (Madison, WI). After adding each solution to a Master mix, 16.5μl of Master mix and 3.5μl of DNA was placed into a 0.5μl PCR tube for a total of 20μl into a Thermocycler at 37ºC for one hour. After one hour the product was run on a 2% agarose gel following the same procedures as PCR amplification for ~1 ½ on the EC4000P voltage 90 machine. The gel was placed in a BioRad GelDoc to analyze if insertion had occurred. The samples were kept at -20ºC until ready for sequencing.

Sequencing

A Thermo Scientific Nanodrop 1000 spectrophotometer (Thermo Scientific, Waltham, MA) was used to determine the concentration of purified plasmid DNA and the amount of sample needed for sequencing.

المنارة للاستشارات
www.manaraa.com

The calculation is as follows:

$$\frac{65/ng/\mu l}{\text{Nanndrop Concentration ng}/\ \mu l} = \mu l \text{ added to } 500\mu l \text{ tube}$$

Using the Beckman Coulter GenomeLab DTCS Quick Start Cycle Sequencing Kit (Fullerton, CA), each sample was prepared by adding the calculated amount of DNA and water to equal 10µl into a PCR tube. Each sample was run both forward and reverse. Forward reaction primer T3 (5`ATTAACCCTCACTAAAGGGA-3`) or reverse reaction primer T7 (5`TAATACGACTCACTATAGGG-3`) was added with DTCS Quick Start Master Mix and then placed on an Eppendorf Thermocycler using a 2 minute 96ºC hot start followed by 20 seconds 96º C denaturing, 20 seconds at 50ºC annealing, and 4 minute 60ºC extension for 31 cycles. After thermocycling procedure was achieved, the reactions were stopped with the addition of stop reaction which includes 2µl Sodium Acetate (pH5.2), 2µl 100mM Na_2EDTA, 1µl 20mg/ml glycogen. After the Stop Reaction was added, 60µl of cold 95% ethanol was mixed with each sample separately and mixed thoroughly. Each sample was immediately placed on an ice block and centrifuged at 14,000rpm for 15 minutes. After centrifugation, the 95% ethanol was removed and the pellet remained. The pellet went through a wash with 200µl of cold 70% ethanol and centrifuged for two minutes at 14,000rpm. After washing twice with 200µl of cold 70% ethanol the sample was then air dried. 40µl of SLS was added for resuspension. Each sample is loaded into a 96- well sequencer plate with one drop of mineral oil added to

المنارة للاستشارات
www.manaraa.com

each well. Samples were sequenced on a Beckman Coulter CEQ 8000, the sample sequences were analyzed using Lasergene EditSeq and forward and reverse sequences were aligned using Lasergene SeqMan to form a consensus. Each sample sequences were run on NCBI Blast to compare each unknown sequence to the NCBI Genbank database to identify the organism.

Real-Time PCR Procedures

Primer Design

Species-specific primer pairs were used for the following fungi: Acremonium strictum, Aspergillus niger, Aspergillus versicolor, Aspergillus terreus, Aspergillus fumigatus, Chaetomium globosum, Trichoderma viride, Trichoderma harzianum, Stachybotrys chartatum, Penicillium purpurogenum, and Paecilomyces variotii. These primers were designed by EPA scientists and listed on the following website: http://www.epa.gov/microbes/moldtech.htm. In addition, primers were designed for A. niger, A.terreus, and A. versicolor. Five sequences were obtained for each fungus from NCBI Genbank and using Lasergene MegAlign each sequence set were aligned to form a consensus sequence for each species. The consensus sequence of A. niger was compared to A. versicolor and A. terreus to find regions unique to each species. Forward primers were selected that were unique to each species with a single reverse primer that matched all three species. Conditions were met for each primer for real-time PCR which included: amplicon size (140-160bp); primer size within 25-28 base pairs (bp) ending in G or C, and at least 40-60% GC content. Once primers were selected, they were checked in Primer Select tools for primer dimers and hairpin loops.

المنارة للاستشارات
www.manaraa.com

Primers were tested by conventional PCR amplification to ensure both forward and reverse primers reacted to selected species and not to others. Sample reactions contained 10µl of iQ Supermix (Biorad, Hercules, CA), 0.5 µl or 2µl forward primer, 0.5µl or 2µl reverse primer, 8µl of sterile water, and 1µl of DNA template. The thermocycler protocol included an initial denaturation at 95°C for 2 minutes, followed by 40 cycles of a 15 second 95°C denaturation, 30 second annealing at 60°C, and a 72°C for 30 second extension. Finally, 10µl of the amplified sample and 2µl of loading dye was loaded on a 2% agarose gel containing GelRed nucleic acid gel stain to visualize band formation if primers were specific for targeted fungi.

Real-Time qPCR

Quantitative Polymerase Chain Reaction (qPCR) was monitored using a fluorescent marker in real-time to determine if specific species of mold were present within building wall material. Real-time qPCR reactions using iCycler iQ™ 96 well PCR plates with Microseal® 'B' Film were performed 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), 3µl sterile water (Sigma), 2µl each forward and reverse (20µM) primer. In addition to the reagents, 3µl of sample DNA was added to each well. 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. To determine the concentration range of each species, a standard curve was developed by using the reaction protocol, which 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. Also a 10-

المنارة للاستشارات
www.manaraa.com

fold dilution of pure culture genomic DNA included a 20µl reaction containing 10µl 2x iQ™ SYBR® Green Supermix (Bio-Rad, Hercules, CA), 5µl sterile water (Sigma), 2µl each forward and reverse (20µM) primer, and 1µl of diluted pure culture genomic DNA into each well and analyzed using iQ™5 Real-Time PCR Detection System (Bio-Rad, Hercules, CA). After the standard curve on each plate was used to estimate the concentration, real-time detection using wall construction materials was monitored during annealing for all material samples which required two 96-well plates for each of the different species.

After amplification, C_T (threshold cycle) values and 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 omitted. Each C_T value that was within the standard curve values were converted to nannograms of genomic DNA per milligram of each material. The results of each conversion were then averaged for each set of treatments. The average concentration of each type of material was graphed into pie charts to show concentration values of each organism contained within the material.

Statistical Analysis

After converting each sample set to nannograms of genomic DNA per milligram of material, statistical differences were determined for each organism found in the different building material. ANOVA test was used to determine if the means of the different building wall materials were significantly different using SAS 9.3. The software also provided the means for comparison of both above and below the water line for the wet building materials.

للاستشارات المنارة
www.manaraa.com

CHAPTER III

RESULTS AND DISCUSSIONS

A total of 168 samples were extracted and examined from the flood unit consisting of building materials that were obtained from Tuskegee, AL. The materials consisting of one hundred thirty-two 'Wet' material samples including solid wood stud (38mm x 89mm x 2.44mm southern yellow pine), vinyl siding, Tyvec housewrap, plywood sheathing, fiberglass batt insulation (R-13), and gypsum wallboard were sent to Mississippi State University for mold identification. In addition to the wet samples, 'Dry' samples were removed seven months later which included thirty six samples consisting of solid wood stud and gypsum wallboard.

Isolation and Identification of Mold from Building Materials

In this investigation a total of nineteen fungi were identified from both wet and dry building materials within the flood unit after flooding. There were eleven different fungal species that were identified by culturing and sequencing including *Aspergillus niger*, *Aspergillus terreus*, *Aspergillus versicolor*, *Chaetomium globosum*, *Cladosporium sp., Penicillium chryogenum*, *Pencillium decumbens*, *Penicillium purpurogenum, Trichoderma viride, Trichoderma reesei, Trichoderma harzianum* and eight mold species identified by cloning including *Acremonium alternatum*, *Alternaria alternata*, *Acremonium strictum*, *Fusarium solani, Fusarium oxysporum, Pestalotiopsis maculans*,

www.manaraa.com

Phoma herbarum, and *Rhizocotonia solani*. Also identified from cloning were the basidiomycetes *Tilletiopsis albescens* and *Trametes versicolor* on the gypsum wallboard. The mold species that were identified by using both culturing and cloning procedures on different wet and dry building materials are shown in Table 3.1.

Table 3.1 Mold species identified from wet and dry building materials. (+ means present and – means not present).

Fungi Species	Wet Gypsum Board	Dry Gypsum Board	Wet Batt Insulation	Wet Wood Sheathing	Wet Wood Stud	Dry Wood Stud	Wet Vinyl Siding
Acremonium strictum	+	-	+	-	-	-	-
Aspergillus fumigatus	-	+	-	-	-	+	-
Aspergillus niger	+	+	+	-	-	+	+
Aspergillus terreus	+	-	+	-	-	-	-
Aspergillus versicolor	-	-	+	-	-	-	-
Chaetomium globosum	+	-	-	+	-	+	+
Cladosporium sp.	+	-	-	-	-	-	-
Fusarium oxysporum	-	+	+	-	-	-	-
Fusarium solani	-	-	+	-	-	-	-
Paecilomyces variotii	-	+	-	-	-	+	-
Penicillium sp.	-	+	-	-	-	-	-
Penicillium purpurogenum	-	-	-	+	-	+	-
Pestalotiopsis/Pestalocia	-	-	+	-	-	-	-
Phoma herbarum	-	-	+	-	-	-	-
Rhizoctonia solani	-	-	-	+	-	-	-
Stachybotrys chartarum	-	+	-	-	-	+	-
Trichoderma harzianum	-	-	-	+	-	-	-
Trichoderma reesei	+	-	-	+	+	-	-
Trichoderma viride	+	-	-	+	-		

There were seven different species of fungi identified from the wet gypsum wallboard, eight from the batt insulation, six from the wood sheathing, and two from the vinyl siding (Table 3.1). In addition to the wet samples, there were six mold species

المنارة للاستشارات
www.manaraa.com

identified from the dry gypsum wallboard and the dry wood stud. The organisms that were identified more frequently in both the wet gypsum wallboard, vinyl siding and dry wood stud samples were *Aspergillus niger* and *Chaetomium globosum*. Additionally, the mold species *Acremonium strictum*, *Aspergillus terreus, Aspergillus versicolor, Fusarium solani*, *Pestalotiopsis sp.,* and *Phoma sp.* were commonly found on the batt insulation using both culturing and cloning procedures. *Aspergillus fumigatus, Paecilomyces variotii,* and *Stachybotrys chartarum* were identified on the dry gypsum wallboard and dry wood stud. It should be noted that *Stachybotrys chartarum* can still be viable and produce mycotoxins on dried materials (Dumon et al. 2009). Finally, the mold isolates *Cladosporium sp.* and *Penicillium sp*. were only identified on the wet gypsum wallboard. It is interesting to note that the three cellulolytic types of *Trichoderma spp*. molds and *Rhizoctonia solani* were found mainly on cellulose-based materials including wet gypsum wallboard and wall sheathing but not on the fiberglass batt insulation. A comparison of species separated by above and below the water exposure is given in Table 3.2.

المنارة للاستشارات
www.manaraa.com

Table 3.2 Mold identified from wall materials separated into exposure above the flood water line and exposure below the flood water line.(+ means present and – means not present)

Fungi Species	Wet Materials Above Water Line	Wet Materials Below Water Line	Dry Materials Above Water Line	Dry Materials Below Water Line
Acremonium strictum	+	+	-	-
Aspergillus fumigatus	-	-	+	+
Aspergillus niger	+	+	+	-
Aspergillus terreus	-	+	-	-
Aspergillus versicolor	+	-	-	-
Chaetomium globosum	+	+	-	+
Cladosporium sp.	-	+	-	-
Fusarium oxysporum	+	+	+	-
Fusarium solani	+	-	-	-
Paecilomyces variotii	-	-	+	-
Penicillium sp.	-	-	-	+
Penicillium purpurogenum	-	+	+	-
Pestalotiopsis/Pestalocia sp.	+	-	-	-
Phoma herbarum	+	-	-	-
Rhizoctonia solani	-	+	-	-
Stachybotrys chartarum	-	-	+	+
Trametes versicolor	+	-	-	-
Trichoderma harzianum	+	+	-	-
Trichoderma reesei	+	+	-	-
Trichoderma viride	+	+	-	-

There was a total of twenty different species of mold present within the flood unit after flooding. Twelve species of fungi were present on the wet materials above the water line after exposure to flood waters, and eleven species of fungi were present below the water line. *Acremonium strictum, Aspergillus niger, Chaetomium globosum, Fusarium oxysporum, Trichoderma harzianum, Trichoderma reesei,* and *Trichoderma viride* were identified both above and below the water line on the wet materials. It is also worth noting that the species *Aspergillus versicolor, Fusarium solani, Pestalotiopsis/Pestalocia sp., Phoma herbarum,* and *Trametes versicolor* were only identified above the water line after exposure, and *Cladosporium sp., Penicillium purpurogenum*, and *Rhizoctonia solani*

www.manaraa.com

were only found below the water line on the wet materials. Six species of fungi were identified on the dry materials located above the water line, and four species were located below the water line. The species *Aspergillus fumigatus* and *Stachybotrys chartarum* were identified more frequently both above and below the water line on the dry materials compared to the other fungal species. *Aspergillus niger, Fusarium oxysporum, Paecilomyces variotii,* and *Penicillium purpurogenum* were located above the water line after exposure, and the species *Chaetomium globosum* and *Penicillium sp.* were only identified below the water level. Therefore, it can be determined that mold species were identified more frequently above the water line than below the water line for both the wet and dry building material samples.

Real-Time PCR

Using real-time PCR, seven mold species including Aspergillus fumigatus, Aspergillus niger, Aspergillus terreus, Aspergillus versicolor, Chaetomium globosum, Paecilomyces variotii, and Stachybotrys chartarum were identified on wet building materials consisting of fiberglass batt insulation, gypsum wallboard, wood sheathing, solid wood stud, house wrap, and vinyl siding located both above and below the water line. In addition to the wet materials, the dry materials including wood stud and gypsum wallboard were analyzed for mold quantification using real-time PCR.

المنارة للاستشارات
www.manaraa.com

Aspergillus fumigatus

Aspergillus fumigatus is a thermotolerant fungi often found world-wide in indoor air. The colonies of *Aspergillus fumigatus*, consist of dense velvety texture of dark green conidiophores intermixed with aerial hyphae bearing conidiophores. *Aspergillus fumigatus* has been found on warm, wet building materials such as wallpaper and in humidifier systems (Samson et al. 2010). This species produces the toxin gliotoxin and is the most important human pathogen causing a mycetoma or fungal ball in lungs (Samson et al. 2010). The DNA concentrations of *Aspergillus fumigatus* was determined using real-time PCR of each wall construction material and converted to the amount of fungal DNA in nannograms per weight of the material sample (Table 3.3).

Table 3.3 Real-time PCR results of *Aspergillus fumigatus* DNA in nannograms per weight of material

Sample Code	Material Weight (mg)	Extraction volume	Amount µL used	Real Time (ng)	Average Reps	(ng)DNA/(mg) Material	Average DNA/Material
B1AP1	100	80	3 µL	8.02E-03		2.14E-03	
B1AP2	100	80	3 µL	1.49E-03	3.20E-03	3.97E-04	8.52E-04
B1AP3	100	80	3 µL	7.71E-05		2.06E-05	
B1BP1	140	80	3 µL	0.00E+00		0.00E+00	
B1BP2	140	80	3 µL	0.00E+00	2.95E-02	0.00E+00	5.62E-03
B1BP3	140	80	3 µL	8.85E-02		1.69E-02	
B1AN1	50	80	3 µL	4.43E-04		2.36E-04	
B1AN2	50	80	3 µL	3.50E-05	1.64E-04	1.87E-05	8.74E-05
B1AN3	50	80	3 µL	1.38E-05		7.36E-06	
B1BN1	100	80	3 µL	2.28E-01		6.08E-02	
B1BN2	100	80	3 µL	1.41E+00	6.82E-01	3.76E-01	1.82E-01
B1BN3	100	80	3 µL	4.09E-01		1.09E-01	
B1AF1	50	80	3 µL	5.27E-05		2.81E-05	
B1AF2	50	80	3 µL	4.09E-05	3.64E-05	2.18E-05	1.94E-05

المنارة للاستشارات
www.manaraa.com

Table 3.3 (continued)

B1AF3	50	80	3 µL	1.56E-05		8.32E-06	
B1BF1	100	80	3 µL	0.00E+00		0.00E+00	
B1BF2	100	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
B1BF3	100	80	3 µL	0.00E+00		0.00E+00	
G3AS1	200	80	3 µL	8.26E-05		1.10E-05	
G3AS2	200	80	3 µL	1.41E-04	7.87E-05	1.88E-05	1.05E-05
G3AS3	200	80	3 µL	1.25E-05		1.67E-06	
G3BS1	250	80	3 µL	0.00E+00		0.00E+00	
G3BS2	250	80	3 µL	2.69E-02	1.11E-02	2.87E-03	1.18E-03
G3BS3	250	80	3 µL	6.40E-03		6.83E-04	
G3AR1	250	80	3 µL	0.00E+00		0.00E+00	
G3AR2	250	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G3AR3	250	80	3 µL	0.00E+00		0.00E+00	
G3BR1	300	80	3 µL	1.23E-02		1.09E-03	
G3BR2	300	80	3 µL	1.48E-06	4.10E-03	1.32E-07	3.65E-04
G3BR3	300	80	3 µL	8.12E-06		7.22E-07	
G3AM1	500	80	3 µL	4.45E-06		2.37E-07	
G3AM2	500	80	3 µL	2.22E-05	8.88E-06	1.18E-06	4.74E-07
G3AM3	500	80	3 µL	0.00E+00		0.00E+00	
G3BM1	500	80	3 µL	0.00E+00		0.00E+00	
G3BM2	500	80	3 µL	4.14E-05	1.38E-05	2.21E-06	7.36E-07
G3BM3	500	80	3 µL	0.00E+00		0.00E+00	
G4AS1	200	80	3 µL	4.39E-06		5.85E-07	
G4AS2	230	80	3 µL	1.33E-03	4.45E-04	1.54E-04	5.16E-05
G4AS3	200	80	3 µL	0.00E+00		0.00E+00	
G4BS1	210	80	3 µL	8.92E-03		1.13E-03	
G4BS2	200	80	3 µL	3.29E-02	1.86E-02	4.39E-03	2.47E-03
G4BS3	200	80	3 µL	1.41E-02		1.88E-03	
G4AR1	100	80	3 µL	0.00E+00		0.00E+00	
G4AR2	100	80	3 µL	0.00E+00	9.70E-04	0.00E+00	2.59E-04
G4AR3	100	80	3 µL	2.91E-03		7.76E-04	
G4BR1	200	80	3 µL	1.05E-04		1.40E-05	
G4BR2	200	80	3 µL	0.00E+00	3.69E-05	0.00E+00	4.92E-06
G4BR3	200	80	3 µL	5.68E-06		7.57E-07	
G4AM1	300	80	3 µL	2.20E-06		1.96E-07	
G4AM2	300	80	3 µL	1.18E-06	1.13E-06	1.05E-07	1.00E-07
G4AM3	320	80	3 µL	0.00E+00		0.00E+00	
G4BM1	500	80	3 µL	0.00E+00		0.00E+00	
G4BM2	520	80	3 µL	8.56E-05	3.06E-05	4.39E-06	1.57E-06
G4BM3	500	80	3 µL	6.09E-06		3.25E-07	

المنارة للاستشارات
www.manaraa.com

Table 3.3 (continued)

G2TS1	150	80	3 µL	2.48E-04		4.41E-05	
G2TS2	160	80	3 µL	0.00E+00	1.86E-04	0.00E+00	3.31E-05
G2TS3	150	80	3 µL	3.11E-04		5.53E-05	
G2BS1	160	80	3 µL	1.07E-02		1.78E-03	
G2BS2	150	80	3 µL	0.00E+00	8.67E-03	0.00E+00	1.50E-03
G2BS3	150	80	3 µL	1.53E-02		2.72E-03	
G2TR1	100	80	3 µL	1.70E-02		4.53E-03	
G2TR2	100	80	3 µL	0.00E+00	5.67E-03	0.00E+00	1.51E-03
G2TR3	100	80	3 µL	0.00E+00		0.00E+00	
G2BR1	100	80	3 µL	0.00E+00		0.00E+00	
G2BR2	80	80	3 µL	2.10E-04	3.16E-04	7.00E-05	1.05E-04
G2BR3	80	80	3 µL	7.39E-04		2.46E-04	
G2TM1	3150	80	3 µL	0.00E+00		0.00E+00	
G2TM2	100	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G2TM3	200	80	3 µL	0.00E+00		0.00E+00	
G2BM1	170	80	3 µL	0.00E+00		0.00E+00	
G2BM2	280	80	3 µL	0.00E+00	1.41E-06	0.00E+00	1.88E-07
G2BM3	200	80	3 µL	4.22E-06		5.63E-07	
W7AS1	100	80	3 µL	5.03E-06		1.34E-06	
W7AS2	100	80	3 µL	2.62E-06	5.04E-06	6.99E-07	1.15E-06
W7AS3	140	80	3 µL	7.46E-06		1.42E-06	
W7BS1	50	80	3 µL	0.00E+00		0.00E+00	
W7BS2	50	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
W7BS3	40	80	3 µL	0.00E+00		0.00E+00	
W7AR1	40	80	3 µL	8.19E-06		5.46E-06	
W7AR2	80	80	3 µL	2.64E-06	4.37E-06	8.80E-07	2.37E-06
W7AR3	80	80	3 µL	2.29E-06		7.63E-07	
W7BR1	50	80	3 µL	0.00E+00		0.00E+00	
W7BR2	60	80	3 µL	0.00E+00	5.77E-03	0.00E+00	3.08E-03
W7BR3	50	80	3 µL	1.73E-02		9.23E-03	
I7A1	40	80	3 µL	4.10E-06		2.73E-06	
I7A2	40	80	3 µL	0.00E+00	2.68E-06	0.00E+00	1.79E-06
I7A3	40	80	3 µL	3.95E-06		2.63E-06	
I7B1	60	80	3 µL	1.47E-02		6.53E-03	
I7B2	50	80	3 µL	2.02E-02	1.72E-02	1.08E-02	8.76E-03
I7B3	50	80	3 µL	1.68E-02		8.96E-03	
V7A1	400	80	3 µL	0.00E+00		0.00E+00	
V7A2	400	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
V7A3	400	80	3 µL	0.00E+00		0.00E+00	
V7B1	400	80	3 µL	0.00E+00		0.00E+00	

المنارة للاستشارات
www.manaraa.com

Table 3.3 (continued)

V7B2	400	80	3 µL	0.00E+00	4.10E-06	0.00E+00	3.64E-07
V7B3	300	80	3 µL	1.23E-05		1.09E-06	
W4AS1	80	80	3 µL	0.00E+00		0.00E+00	
W4AS2	90	80	3 µL	2.11E-03	1.49E-03	6.25E-04	2.36E-04
W4AS3	760	80	3 µL	2.37E-03		8.32E-05	
W4BS1	900	80	3 µL	1.17E-02		3.47E-04	
W4BS2	76	80	3 µL	2.43E-03	4.97E-03	8.53E-04	4.53E-04
W4BS3	130	80	3 µL	7.77E-04		1.59E-04	
W4BR1	150	80	3 µL	0.00E+00		0.00E+00	
W4AR2	90	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
W4AR3	70	80	3 µL	0.00E+00		0.00E+00	
W4BR1	40	80	3 µL	9.12E-04		6.08E-04	
W4BR2	65	80	3 µL	3.07E-03	3.71E-03	1.26E-03	1.89E-03
W4BR3	50	80	3 µL	7.15E-03		3.81E-03	
W4AM1	60	80	3 µL	7.41E-06		3.29E-06	
W4AM2	50	80	3 µL	3.95E-06	3.79E-06	2.11E-06	1.80E-06
W4AM3	40	80	3 µL	0.00E+00		0.00E+00	
W4BM1	53	80	3 µL	0.00E+00		0.00E+00	
W4BM2	50	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
W4BM3	66	80	3 µL	0.00E+00		0.00E+00	
G1AS1	120	80	3 µL	1.08E-02		2.40E-03	
G1AS2	170	80	3 µL	0.00E+00	4.81E-03	0.00E+00	9.61E-04
G1AS3	200	80	3 µL	3.62E-03		4.83E-04	
G1BS1	200	80	3 µL	0.00E+00		0.00E+00	
G1BS2	300	80	3 µL	4.57E-03	4.30E-03	4.06E-04	2.84E-04
G1BS3	500	80	3 µL	8.34E-03		4.45E-04	
G1AR1	62	80	3 µL	0.00E+00		0.00E+00	
G1AR2	75	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G1AR3	100	80	3 µL	0.00E+00		0.00E+00	
G1BR1	97	80	3 µL	0.00E+00		0.00E+00	
G1BR2	100	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G1BR3	150	80	3 µL	0.00E+00		0.00E+00	
G1AM1	500	80	3 µL	0.00E+00		0.00E+00	
G1AM2	430	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G1AM3	427	80	3 µL	0.00E+00		0.00E+00	
G1BM1	450	80	3 µL	0.00E+00		0.00E+00	
G1BM2	360	80	3 µL	2.06E-06	1.80E-06	1.53E-07	1.06E-07
G1BM3	540	80	3 µL	3.35E-06		1.65E-07	
S1AF1	50	80	3 µL	2.69E-04		1.43E-04	
S1AF2	50	80	3 µL	3.40E-05	1.55E-04	1.81E-05	8.27E-05

www.manaraa.com

Table 3.3 (continued)

S1AF3	50	80	3 µL	1.62E-04		8.64E-05	
S1AM1	50	80	3 µL	5.14E-01		2.74E-01	
S1AM2	50	80	3 µL	5.72E-02	1.90E-01	3.05E-02	1.02E-01
S1AM3	60	80	3 µL	2.67E-06		1.19E-06	
S1AR1	50	80	3 µL	1.59E-04		8.48E-05	
S1AR2	50	80	3 µL	1.57E-05	6.08E-05	8.37E-06	3.24E-05
S1AR3	50	80	3 µL	7.67E-06		4.09E-06	
S2BF1	50	80	3 µL	0.00E+00		0.00E+00	
S2BF2	60	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
S2BF3	50	80	3 µL	0.00E+00		0.00E+00	
S2BM1	60	80	3 µL	1.42E-05		6.31E-06	
S2BM2	50	80	3 µL	2.57E-05	1.73E-05	1.37E-05	8.44E-06
S2BM3	60	80	3 µL	1.19E-05		5.29E-06	
S2BR1	50	80	3 µL	0.00E+00		0.00E+00	
S2BR2	50	80	3 µL	0.00E+00	1.08E-04	0.00E+00	5.78E-05
S2BR3	50	80	3 µL	3.25E-04		1.73E-04	
G3AF1	200	80	3 µL	0.00E+00		0.00E+00	
G3AF2	100	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G3AF3	200	80	3 µL	0.00E+00		0.00E+00	
G3AM1	200	80	3 µL	0.00E+00		0.00E+00	
G3AM2	200	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G3AM3	200	80	3 µL	0.00E+00		0.00E+00	
G3AR1	100	80	3 µL	0.00E+00		0.00E+00	
G3AR2	100	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G3AR3	100	80	3 µL	0.00E+00		0.00E+00	
G4BF1	100	80	3 µL	0.00E+00		0.00E+00	
G4BF2	200	80	3 µL	1.10E-02	3.67E-03	1.47E-03	4.89E-04
G4BF3	200	80	3 µL	0.00E+00		0.00E+00	
G4BM1	300	80	3 µL	3.28E-06		2.92E-07	
G4BM2	200	80	3 µL	0.00E+00	1.09E-06	0.00E+00	9.72E-08
G4BM3	200	80	3 µL	0.00E+00		0.00E+00	
G4BR1	100	80	3 µL	8.05E-03		2.15E-03	
G4BR2	100	80	3 µL	2.03E-03	4.49E-03	5.41E-04	1.20E-03
G4BR3	100	80	3 µL	3.39E-03		9.04E-04	

The average concentration of *Aspergillus fumigatus* on each type of material is shown in Figure 3.1.

المنارة للاستشارات
www.manaraa.com

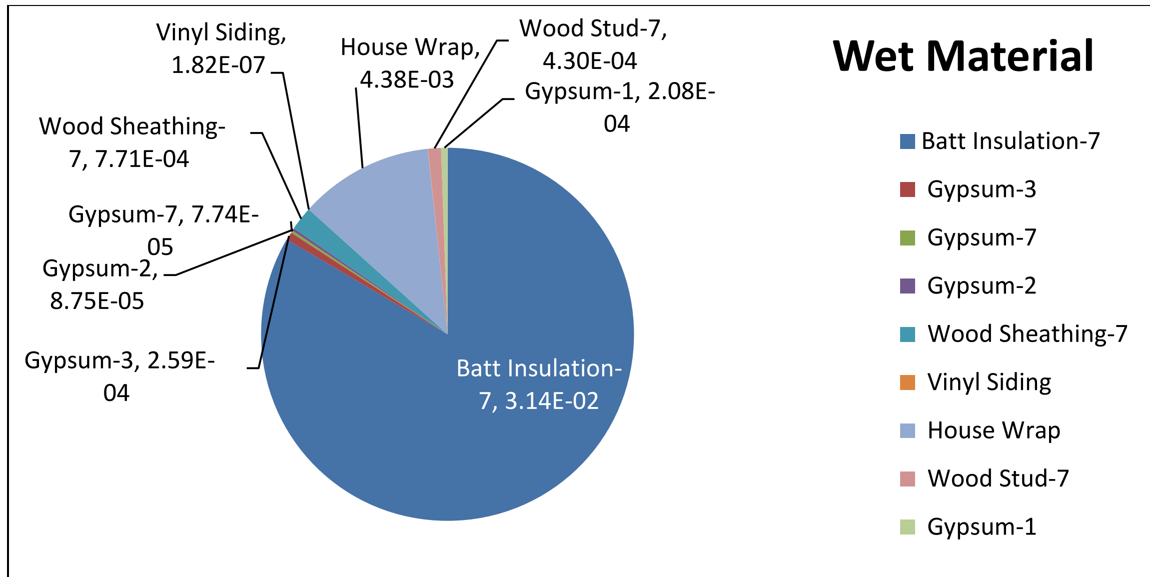

Figure 3.1 The distribution of *Aspergillus fumigatus*, based on averaged DNA concentrations, on the different wet building materials. Samples with a 7 came from the same wall unit.

Aspergillus fumigatus was present on all wall materials in Figure 3.1. The batt insulation contained the highest average concentration of $3.14E^{-02}$ while the vinyl siding ($1.82E^{-07}$) contained the lowest concentration of DNA. There was no statistical difference based on average DNA concentrations among the different wall materials (Table 3.4). The other components of the wall unit, wood stud ($4.30E^{-04}$), batt insulation ($8.73E^{-04}$), wood sheathing ($7.71E^{-04}$), and gypsum-7 ($7.74E^{-05}$), all contained moderate levels of *A. fumigatus*.

المنارة للاستشارات
www.manaraa.com

Table 3.4 Statistical analysis of each wet building material. (Means with same letter are not significantly different).

ANOVA Statistical Difference			
Tukey Grouping	**Mean**	**N**	**Material**
A	0.03142	18	Batt Insulation
A	0.00438	6	House wrap
A	0.00077	12	Wood sheathing
A	0.00053	18	Gypsum-2
A	0.00046	18	Gypsum-7
A	0.00043	18	Wood stud
A	0.00026	18	Gypsum-3
A	0.00021	18	Gypsum-1
A	0.00000	6	Vinyl Siding

The distribution of *Aspergillus fumigatus* on the individual wall materials are given in Figures 3.2-3.8.

المنارة للاستشارات
www.manaraa.com

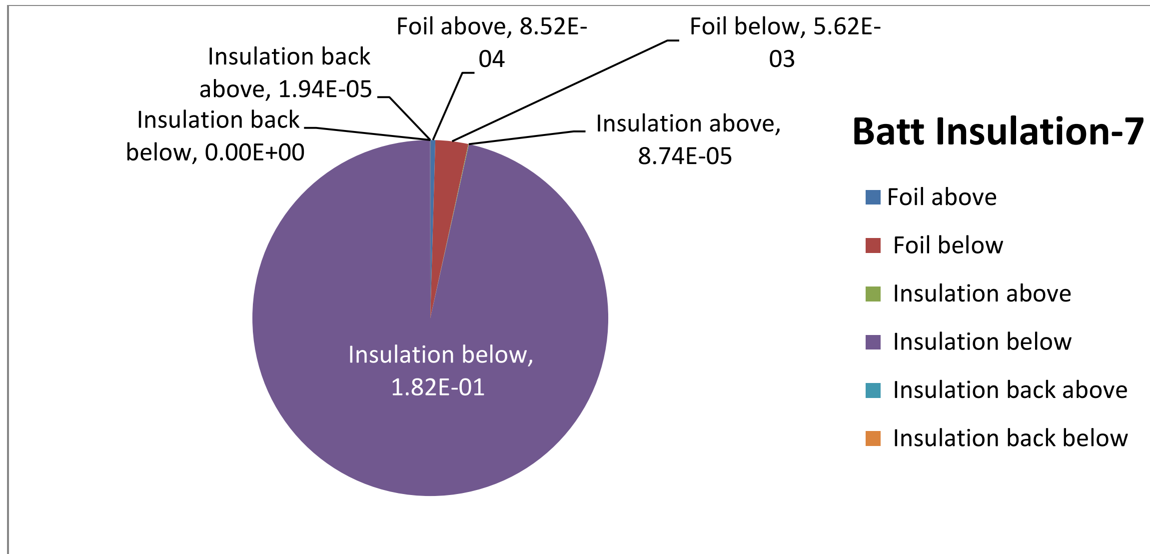

Figure 3.2 The distribution of *Aspergillus fumigatus*, based on DNA concentrations, on the different components of the batt insulation-7.

The distribution of *Aspergillus fumigatus* in the batt insulation (Figure 3.2) indicates that *Aspergillus fumigatus* was found in high concentration of $1.82E^{-01}$ below the water line on the insulation itself (purple). The other batt insulation components including the insulation above ($8.74E^{-05}$), foil below ($5.62E^{-03}$) and above ($8.52E^{-04}$), and insulation back above ($1.94E^{-05}$) the water line supported lower concentrations. Although the highest average concentration was found in the house wrap, the batt insulation below the water line contained the highest single concentration of *A. fumigatus*. The fiberglass batt was separated into foil, the fiberglass insulation(R-13), and insulation backing above and below the water line.

المنارة للاستشارات
www.manaraa.com

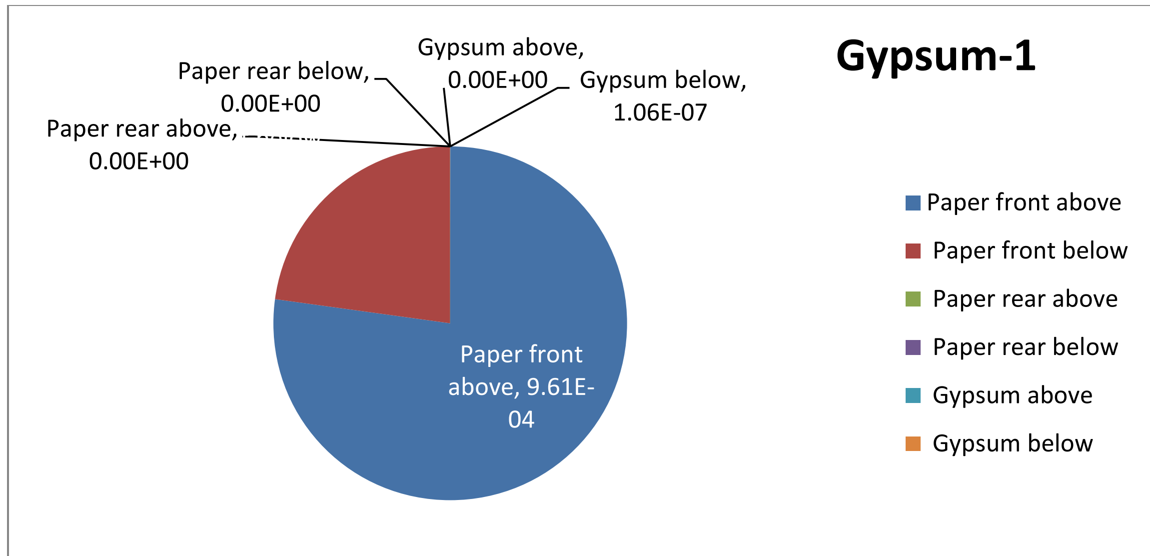

Figure 3.3 The distribution of *Aspergillus fumigatus*, based on DNA concentrations, on the different components of gypsum-1.

Figure 3.3 shows the distribution of *Aspergillus fumigatus* on the gypsum-1 sample. The paper on the front (interior of the house) of the gypsum board above the water line contained the highest concentration of *A. fumigatus* ($9.61E^{-04}$). The paper on the front below the water line contained $2.84E^{-04}$. The other samples including the gypsum itself both above and below the water line, and the paper backing on the rear of the gypsum board both below and above the water line showed no or very low presence of *Aspergillus fumigatus*.

المنارة للاستشارات
www.manaraa.com

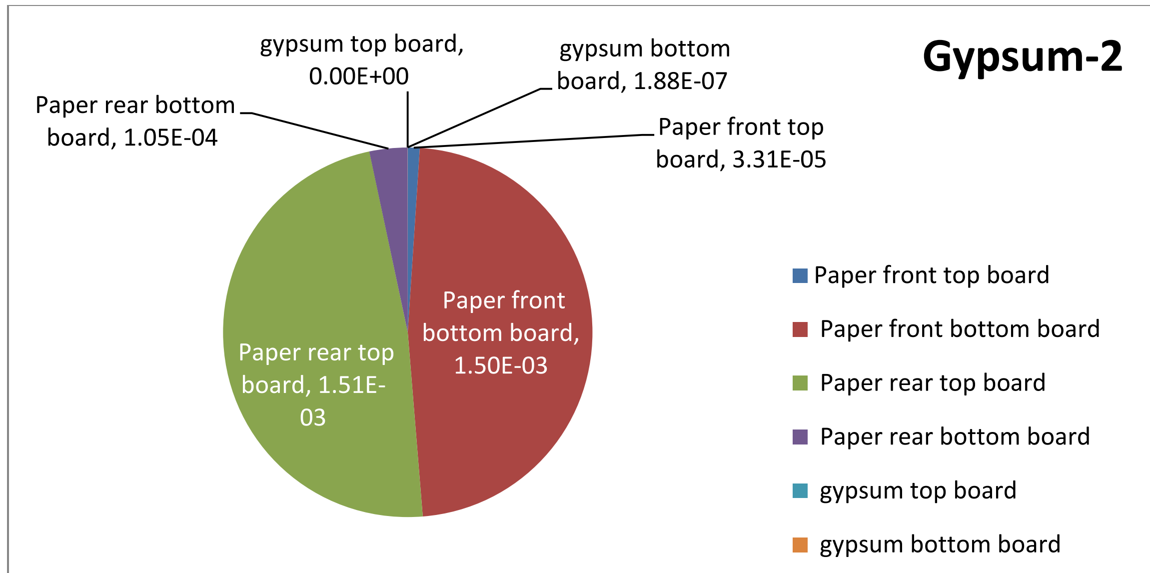

Figure 3.4 The distribution of *Aspergillus fumigatus*, based on DNA concentrations, on the different components of gypsum-2.

Figure 3.4 shows that on the gypsum-2 board *Aspergillus fumigatus* was present in a higher concentration of $1.50E^{-03}$ on the paper at the front bottom of the board while the back paper at the top of the board was the next highest concentrations ($1.51E^{-03}$). The paper at the back bottom of the board ($1.05E^{-04}$) and the paper at the front top ($3.31E^{-05}$) also contained notable levels of the fungal DNA. The gypsum itself contained no or very little of the fungus.

المنارة للاستشارات
www.manaraa.com

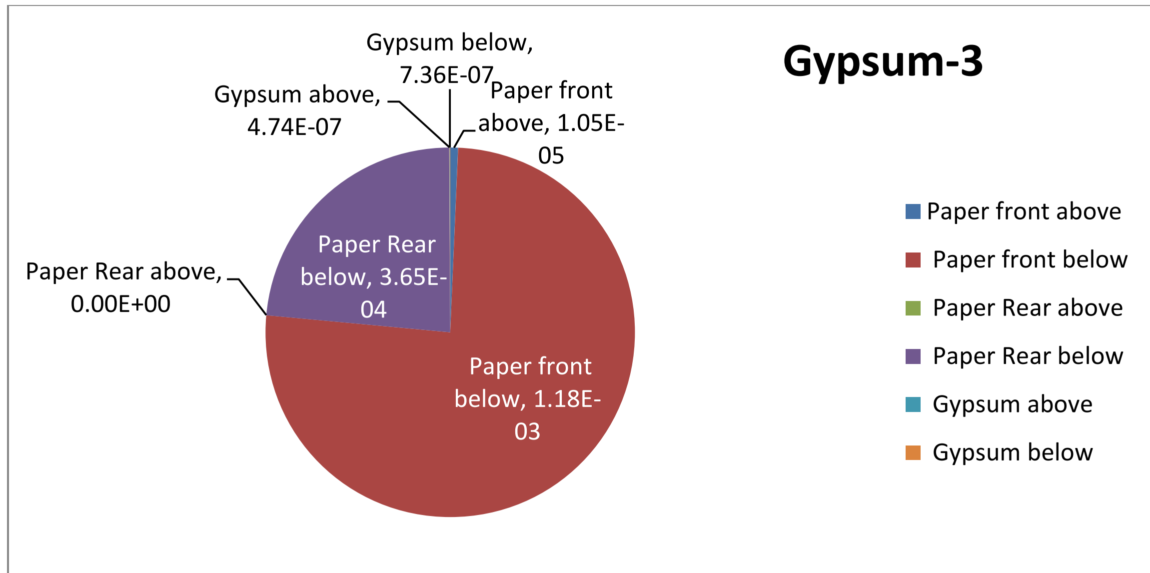

Figure 3.5 The distribution of *Aspergillus fumigatus*, based on DNA concentrations, on the different components of gypsum-3.

Gypsum-3 in Figure 3.5 shows that *Aspergillus fumigatus* was found at a concentration of 1.18E^{-03} below the water line on the paper front compared to a lower concentration of 3.65E^{-04} on the paper rear below the water line. *Aspergillus fumigatus* was found on the gypsum samples; however, the concentration was very low. The distribution of *Aspergillus fumigatus* on the paper above the water line was lower than the concentrations found below the water line.

www.manaraa.com

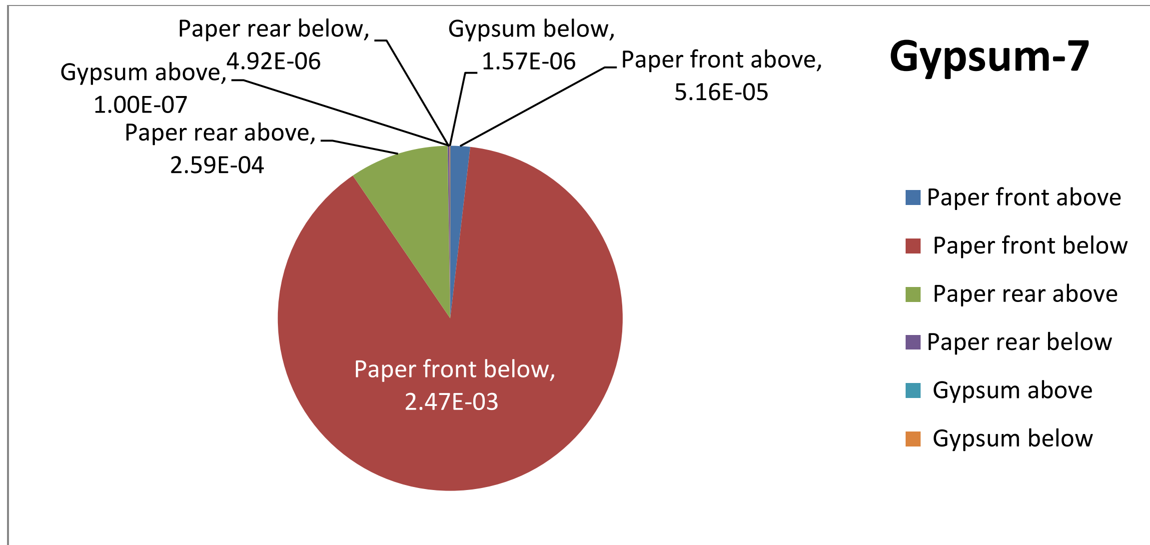

Figure 3.6 The distribution of *Aspergillus fumigatus*, based on DNA concentrations, on the different components of gypsum-7.

Figure 3.6 shows that *Aspergillus fumigatus* was present on gypsum-7 in the highest concentration of $2.47E^{-03}$ on the front paper below the water line, while the front paper above the water line contained a lower concentration of $5.16E^{-05}$. The second highest concentration of *Aspergillus fumigatus* on the gypsum was on the paper rear above the water line with an average concentration of $1.32E^{-04}$. It is possible that more *Aspergillus fumigatus* was present in higher concentrations on paper in the front below the water line compared to the paper located on the rear of the gypsum board because the silt left behind from the flood waters could have been a source of nutrients and inoculum for mold growth to occur.

المنارة للاستشارات
www.manaraa.com

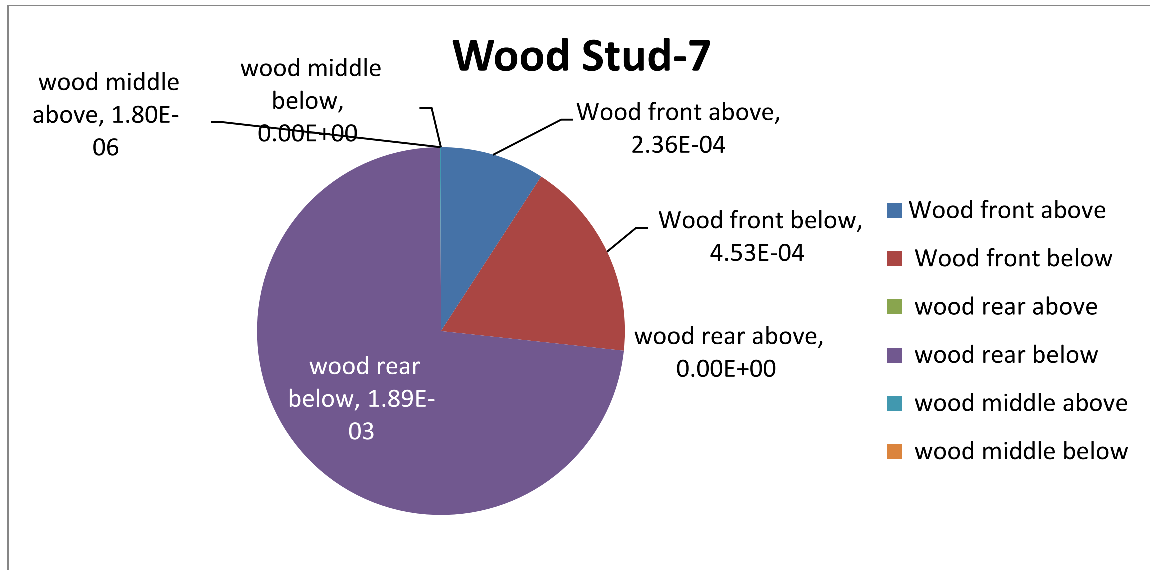

Figure 3.7 The distribution of *Aspergillus fumigatus*, based on DNA concentrations, on the different components of wood stud-7.

Wood stud-7 in Figure 3.7 shows that *Aspergillus fumigatus* was present in a moderately high concentration of $1.89E^{-03}$ on the rear of the wood stud followed by the front of the wood stud ($4.53E^{-04}$) both below the waterline. There was also a notable concentration on the front of the wood stud above the water line ($2.36E^{-04}$). *Aspergillus fumigatus* was present in very low concentrations or absent in the middle of the wood stud. This implies that since *Aspergillus fumigatus* is a hydrophilic fungus, it grows well in environments that offer a high moisture content (Latge 1999).

المنارة للاستشارات
www.manaraa.com

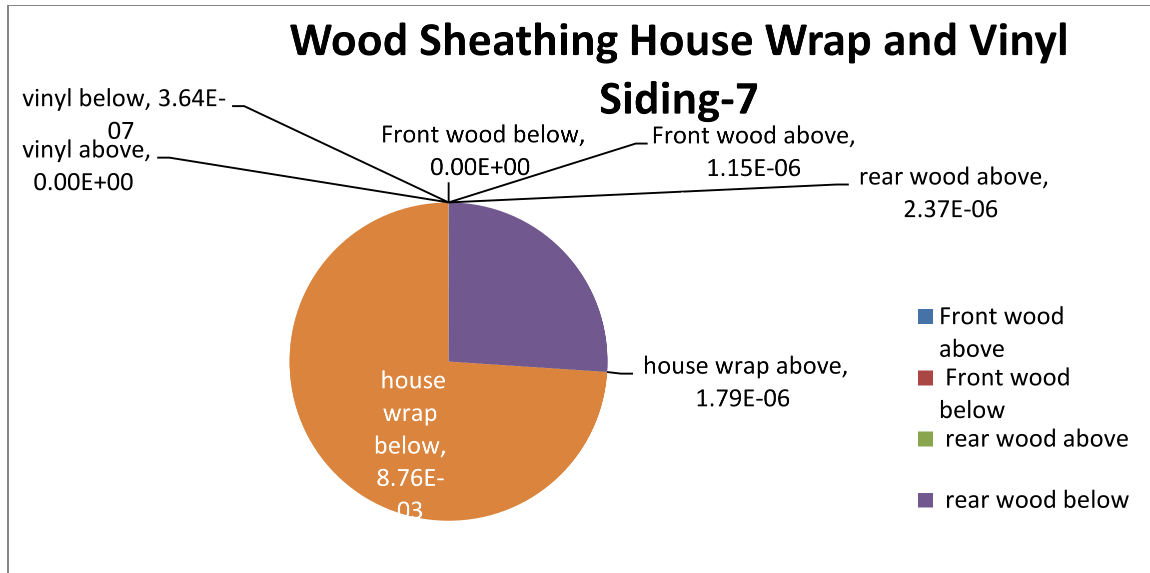

Figure 3.8 The distribution of *Aspergillus fumigatus*, based on DNA concentrations, on the different components of the wood sheathing, plus the house wrap, and vinyl siding-7.

In Figure 3.8 *Aspergillus fumigatus* is shown to be present in a concentration of $8.76E^{-03}$ on the house wrap below the water line and a concentration of $3.08E^{-03}$ on the rear side of the wood sheathing located below the water line. *Aspergillus fumigatus* was found to be present in much lower concentrations on the vinyl below the water line ($3.64E^{-07}$), on the front section of the wood sheathing above the water line ($1.15E^{-06}$), and the rear section of the wood sheathing above the water line ($2.37E^{-06}$). No *Aspergillus fumigatus* was present either on the vinyl siding above the water line or on the front of the wood sheathing below the water line. Table 3.5 shows the analysis of *Aspergillus fumigatus* on the wet building materials both above and below the water line.

للاستشارات المنارة
www.manaraa.com

Table 3.5 The average of *Aspergillus fumigatus* on both above and below the water line on wet building materials.

State	Waterline	Samples	Mean	Std Dev
wet	above	57	0.0001308	0.00044
wet	below	57	0.0108452	0.051914

The statistical anaylsis of the wet building materials above and below the water line shows that *Aspergillus fumigatus* (Table 3.5) was present in a much higher concentration below the water line than above the water line compared to above the water line. The dry samples were examined for *Aspergillus fumigatus* on the wood stud and gypsum wallboard. The different dry materials examined for the mold species both above and below the water line are shown in Figures 3.9- 3.11.

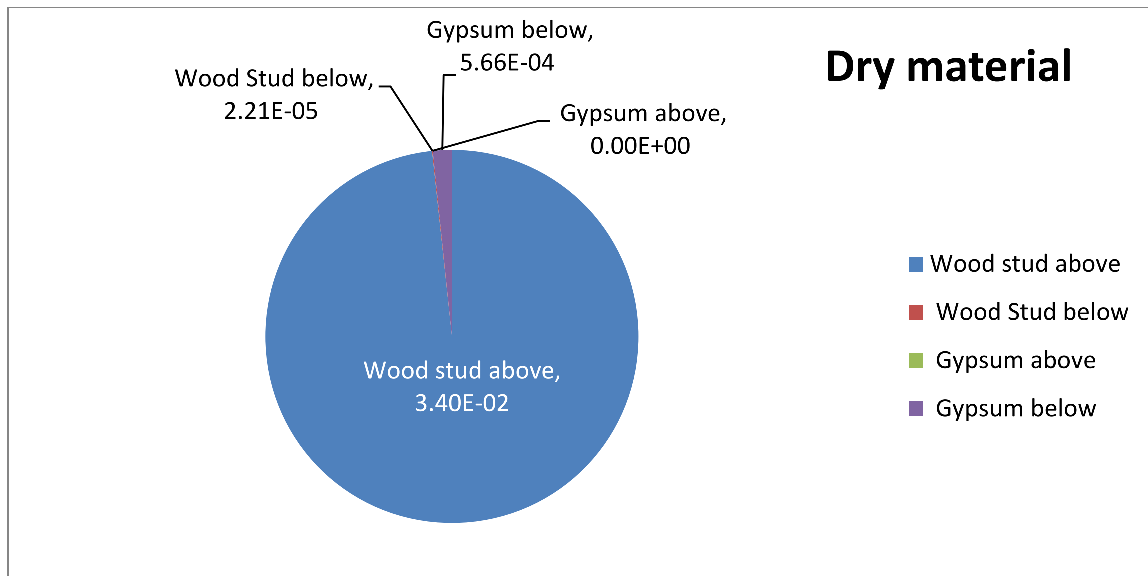

Figure 3.9 The distribution of *Aspergillus fumigatus*, based on DNA concentrations, on the different components of the dry wall material samples.

المنارة للاستشارات
www.manaraa.com

The dry wall materials in Figure 3.9 show that *Aspergillus fumigatus* was present in all materials except for gypsum above the water line; however the dry wood stud above the water line supported the vast majority of this species at a high concentration of $3.40E^{-02}$. The gypsum below the water line ($5.66E^{-04}$) and the wood stud below the water line ($2.21E^{-05}$) also supported notable concentrations of this species. Table 3.6 shows that there was no significant difference in mean concentration between the dry materials.

Table 3.6 The significant difference of *Aspergillus fumigatus* on the dry building materials. (Means with the same letter are not significantly different).

ANOVA Statistical Difference			
Tukey Grouping	**Mean**	**N**	**Material**
A	0.01695	18	Wood stud
A	0.00028	18	Gypsum

The presence of *Aspergillus fumigatus* on the different components of the wall samples, are given in Figures 3.10- 3.11.

المنارة للاستشارات
www.manaraa.com

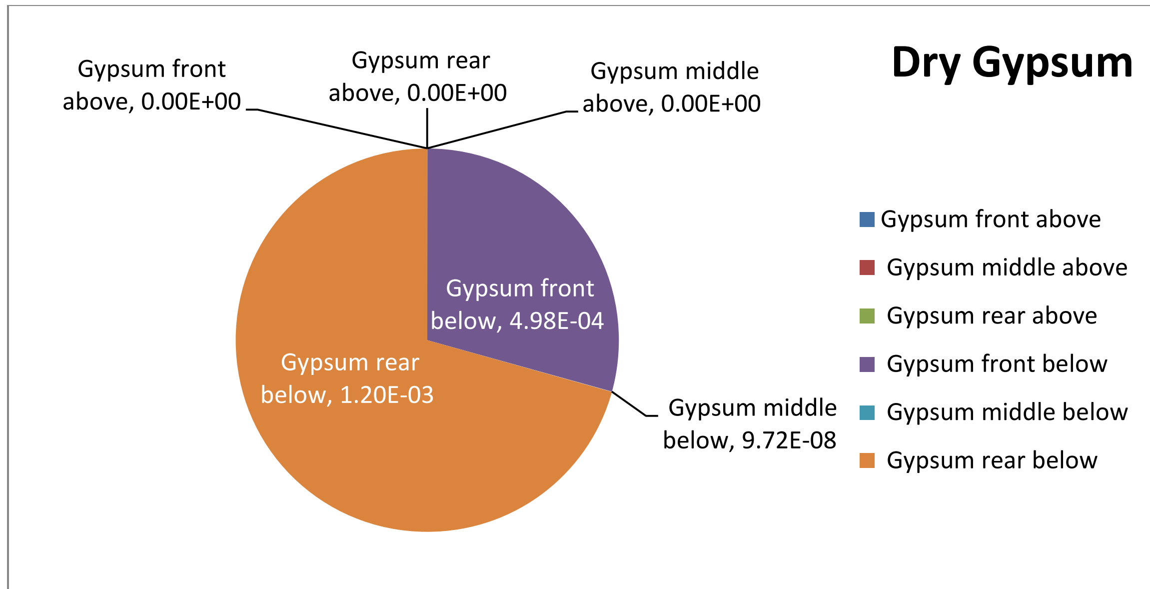

Figure 3.10 The distribution of *Aspergillus fumigatus*, based on DNA concentrations, on the different components of the dry gypsum.

Figure 3.10 shows the distribution of *Aspergillus fumigatus* on the dry gypsum wallboard. *Aspergillus fumigatus* was found in the highest concentration ($1.20E^{-03}$) on the rear paper below the water line. The front paper below the water line contained the second highest concentration of $4.98E^{-04}$. The gypsum itself and the other materials contained no or very little *Aspergillus fumigatus*.

المنارة للاستشارات
www.manaraa.com

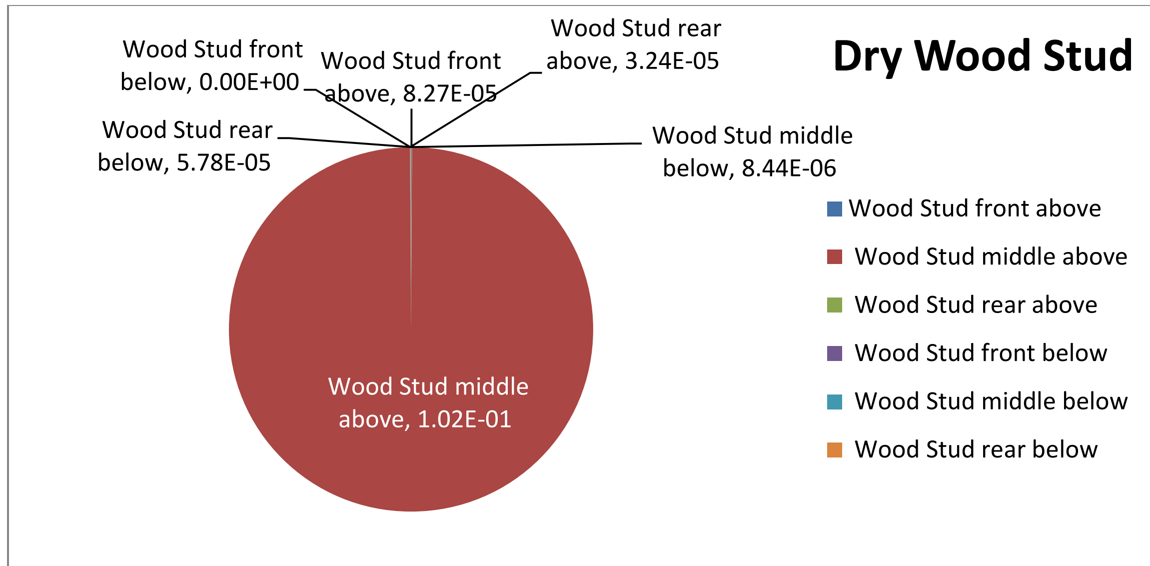

Figure 3.11 The distribution of *Aspergillus fumigatus*, based on DNA concentrations, on the different components of the dry wood stud.

Figure 3.11 shows the distribution of *Aspergillus fumigatus* on the dry wood stud. Surprisingly, the middle section of the 2x4 wood stud above the water line supported a high concentration of this species and made up a large majority of what was detected ($1.02E^{-01}$). The other components including the wood stud middle below the water line ($8.44E^{-06}$), wood stud rear above the water line ($3.24E^{-05}$), the wood stud front above the water line ($8.27E^{-05}$), and wood stud rear below the water line ($5.78E^{-05}$) supported a presence of *Aspergillus fumigatus*, however, it was much lower than the middle section above the water line.

In summary, *Aspergillus fumigatus* was found in the highest concentration of any of the seven fungi measured on the wet batt insulation below the water line (E^{-01}) and on the dry wood stud (E^{-01}). Because this species is an important human pathogen, its presence and concentrations on both the wet and the dry wall materials is a grave

المنارة للاستشارات
www.manaraa.com

concern. Obviously this species thrives in a very moist environment as was documented by its presence primarily below the water line on most all of the wall materials tested. It is a known hydrophilic fungus. It was also one of the few fungi measured that was present in substantial levels on the house wrap, which did not tend to support the growth of the other species. Also noteworthy was its ability to remain in high concentrations on the dry materials seven months after the flood. This is definitely a species that bears closer scrutiny in flood damaged homes in the southern United States.

Aspergillus niger

Aspergillus niger is a halo-tolerant organism which grows well at high temperatures, can tolerate low pH, and low moisture content. *Aspergillus niger* is often found world-wide in indoor environments, but not typically associated with contaminated building materials (Flannigan et al. 2011). *Aspergillus niger* is in the phylum Ascomycota and is a filamentous fungi which produces purple-black, brown-black or black conidia and grows rapidly on all media (Samson et al. 2010). *Aspergillus niger* is the cause of primary cutaneous, pulmonary, and disseminated infection in severely immunocompromised individuals. *Aspergillus niger* can be isolated from soil and decomposing plant material. The DNA concentrations of *Aspergillus niger* was determined using real-time PCR on each wall construction material and converted to the amount of fungal DNA in nannograms per weight of the material sample (Table 3.7). A summary of the average DNA concentrations for each wall materials is shown in Figure 3.12.

المنارة للاستشارات
www.manaraa.com

Table 3.7 Real-time PCR results of *Aspergillus niger* DNA in nannograms per weight of material

Sample Code	Material Weight (mg)	Extraction Volume	Amount µL used	Real Time (ng)	Average Reps	(ng)DNA/(mg) Material	Average DNA/Material
B1AP1	100	80	3 µL	1.01E-05		2.69E-06	
B1AP2	100	80	3 µL	7.63E-06	6.48E-06	2.03E-06	1.73E-06
B1AP3	100	80	3 µL	1.70E-06		4.53E-07	
B1BP1	140	80	3 µL	0.00E+00		0.00E+00	
B1BP2	140	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
B1BP3	140	80	3 µL	0.00E+00		0.00E+00	
B1AN1	50	80	3 µL	2.06E-05		1.10E-05	
B1AN2	50	80	3 µL	3.59E-06	8.48E-06	1.91E-06	4.52E-06
B1AN3	50	80	3 µL	1.25E-06		6.67E-07	
B1BN1	100	80	3 µL	8.47E-06		2.26E-06	
B1BN2	100	80	3 µL	1.18E-05	9.16E-06	3.15E-06	2.44E-06
B1BN3	100	80	3 µL	7.20E-06		1.92E-06	
B1AF1	50	80	3 µL	4.84E-07		2.58E-07	
B1AF2	50	80	3 µL	1.24E-06	5.75E-07	6.61E-07	3.06E-07
B1AF3	50	80	3 µL	0.00E+00		0.00E+00	
B1BF1	100	80	3 µL	0.00E+00		0.00E+00	
B1BF2	100	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
B1BF3	100	80	3 µL	0.00E+00		0.00E+00	
G3AS1	200	80	3 µL	1.02E-07		1.36E-08	
G3AS2	200	80	3 µL	4.50E-06	1.53E-06	6.00E-07	2.05E-07
G3AS3	200	80	3 µL	0.00E+00		0.00E+00	
G3BS1	250	80	3 µL	0.00E+00		0.00E+00	
G3BS2	250	80	3 µL	N/A	2.27E-06	N/A	2.42E-07
G3BS3	250	80	3 µL	4.53E-06		4.83E-07	
G3AR1	250	80	3 µL	0.00E+00		0.00E+00	
G3AR2	250	80	3 µL	2.13E-07	7.10E-08	2.27E-08	7.57E-09
G3AR3	250	80	3 µL	0.00E+00		0.00E+00	
G3BR1	300	80	3 µL	9.25E-07		8.22E-08	
G3BR2	300	80	3 µL	2.41E-07	3.89E-07	2.14E-08	3.45E-08
G3BR3	300	80	3 µL	0.00E+00		0.00E+00	
G3AM1	500	80	3 µL	0.00E+00		0.00E+00	
G3AM2	500	80	3 µL	4.13E-07	2.40E-07	2.20E-08	1.28E-08
G3AM3	500	80	3 µL	3.07E-07		1.64E-08	
G3BM1	500	80	3 µL	3.64E-07		1.94E-08	
G3BM2	500	80	3 µL	4.34E-07	3.99E-07	2.31E-08	2.13E-08

المنارة للاستشارات
www.manaraa.com

Table 3.7 (continued)

G3BM3	500	80	3 μL	4.00E-07		2.13E-08	
G4AS1	200	80	3 μL	1.62E-06		2.16E-07	
G4AS2	230	80	3 μL	1.15E-07	1.70E-06	1.33E-08	2.25E-07
G4AS3	200	80	3 μL	3.35E-06		4.47E-07	
G4BS1	210	80	3 μL	6.69E-06		8.50E-07	
G4BS2	200	80	3 μL	0.00E+00	3.86E-06	0.00E+00	5.01E-07
G4BS3	200	80	3 μL	4.90E-06		6.53E-07	
G4AR1	100	80	3 μL	7.97E-08		2.13E-08	
G4AR2	100	80	3 μL	1.52E-06	2.40E-06	4.05E-07	6.40E-07
G4AR3	100	80	3 μL	5.60E-06		1.49E-06	
G4BR1	200	80	3 μL	2.08E-07		2.77E-08	
G4BR2	200	80	3 μL	4.46E-07	4.64E-07	5.95E-08	6.19E-08
G4BR3	200	80	3 μL	7.39E-07		9.85E-08	
G4AM1	300	80	3 μL	2.10E-06		1.87E-07	
G4AM2	300	80	3 μL	N/A	1.05E-06	N/A	9.33E-08
G4AM3	320	80	3 μL	0.00E+00		0.00E+00	
G4BM1	500	80	3 μL	1.62E-06		8.64E-08	
G4BM2	520	80	3 μL	3.74E-06	1.79E-06	1.92E-07	9.27E-08
G4BM3	500	80	3 μL	0.00E+00		0.00E+00	
G2TS1	150	80	3 μL	0.00E+00		0.00E+00	
G2TS2	160	80	3 μL	0.00E+00	7.87E-07	0.00E+00	1.40E-07
G2TS3	150	80	3 μL	2.36E-06		4.20E-07	
G2BS1	160	80	3 μL	2.53E-06		4.22E-07	
G2BS2	150	80	3 μL	0.00E+00	2.56E-06	0.00E+00	4.46E-07
G2BS3	150	80	3 μL	5.16E-06		9.17E-07	
G2TR1	100	80	3 μL	9.41E-06		2.51E-06	
G2TR2	100	80	3 μL	5.73E-06	6.10E-06	1.53E-06	1.63E-06
G2TR3	100	80	3 μL	3.15E-06		8.40E-07	
G2BR1	100	80	3 μL	3.98E-07		1.06E-07	
G2BR2	80	80	3 μL	6.42E-07	1.29E-06	2.14E-07	4.21E-07
G2BR3	80	80	3 μL	2.83E-06		9.43E-07	
G2TM1	3150	80	3 μL	1.33E-07		1.13E-09	
G2TM2	100	80	3 μL	2.49E-08	1.58E-07	6.64E-09	1.66E-08
G2TM3	200	80	3 μL	3.15E-07		4.20E-08	
G2BM1	170	80	3 μL	6.23E-07		9.77E-08	
G2BM2	280	80	3 μL	1.56E-07	3.90E-07	1.49E-08	5.63E-08
G2BM3	200	80	3 μL	N/A		N/A	
W7AS1	100	80	3 μL	4.62E-07		1.23E-07	
W7AS2	100	80	3 μL	3.32E-07	5.21E-07	8.85E-08	1.19E-07
W7AS3	140	80	3 μL	7.69E-07		1.46E-07	

المنارة للاستشارات
www.manaraa.com

Table 3.7 (continued)

W7BS1	50	80	3 µL	0.00E+00		0.00E+00	
W7BS2	50	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
W7BS3	40	80	3 µL	0.00E+00		0.00E+00	
W7AR1	40	80	3 µL	1.06E-06		7.07E-07	
W7AR2	80	80	3 µL	8.29E-07	7.65E-07	2.76E-07	3.73E-07
W7AR3	80	80	3 µL	4.07E-07		1.36E-07	
W7BR1	50	80	3 µL	0.00E+00		0.00E+00	
W7BR2	60	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
W7BR3	50	80	3 µL	0.00E+00		0.00E+00	
I7A1	40	80	3 µL	0.00E+00		0.00E+00	
I7A2	40	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
I7A3	40	80	3 µL	0.00E+00		0.00E+00	
I7B1	60	80	3 µL	0.00E+00		0.00E+00	
I7B2	50	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
I7B3	50	80	3 µL	0.00E+00		0.00E+00	
V7A1	400	80	3 µL	0.00E+00		0.00E+00	
V7A2	400	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
V7A3	400	80	3 µL	0.00E+00		0.00E+00	
V7B1	400	80	3 µL	0.00E+00		0.00E+00	
V7B2	400	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
V7B3	300	80	3 µL	0.00E+00		0.00E+00	
W4AS1	80	80	3 µL	0.00E+00		0.00E+00	
W4AS2	90	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
W4AS3	760	80	3 µL	0.00E+00		0.00E+00	
W4BS1	900	80	3 µL	0.00E+00		0.00E+00	
W4BS2	76	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
W4BS3	130	80	3 µL	0.00E+00		0.00E+00	
W4AR1	150	80	3 µL	0.00E+00		0.00E+00	
W4AR2	90	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
W4AR3	70	80	3 µL	0.00E+00		0.00E+00	
W4BR1	40	80	3 µL	0.00E+00		0.00E+00	
W4BR2	65	80	3 µL	8.37E-06	2.79E-06	3.43E-06	1.14E-06
W4BR3	50	80	3 µL	0.00E+00		0.00E+00	
W4AM1	60	80	3 µL	0.00E+00		0.00E+00	
W4AM2	50	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
W4AM3	40	80	3 µL	0.00E+00		0.00E+00	
W4BM1	53	80	3 µL	0.00E+00		0.00E+00	
W4BM2	50	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
W4BM3	66	80	3 µL	0.00E+00		0.00E+00	
G1AS1	120	80	3 µL	1.12E-06		2.49E-07	

www.manaraa.com

Table 3.7 (continued)

G1AS2	170	80	3 μL	0.00E+00	4.44E-06	0.00E+00	6.25E-07
G1AS3	200	80	3 μL	1.22E-05		1.63E-06	
G1BS1	200	80	3 μL	0.00E+00		0.00E+00	
G1BS2	300	80	3 μL	6.23E-07	2.08E-07	5.54E-08	1.85E-08
G1BS3	500	80	3 μL	0.00E+00		0.00E+00	
G1AR1	62	80	3 μL	2.91E-07		1.25E-07	
G1AR2	75	80	3 μL	0.00E+00	9.70E-08	0.00E+00	4.17E-08
G1AR3	100	80	3 μL	0.00E+00		0.00E+00	
G1BR1	97	80	3 μL	0.00E+00		0.00E+00	
G1BR2	100	80	3 μL	2.93E-08	1.33E-07	7.81E-09	2.45E-08
G1BR3	150	80	3 μL	3.69E-07		6.56E-08	
G1AM1	500	80	3 μL	2.07E-08		1.10E-09	
G1AM2	430	80	3 μL	3.18E-09	8.47E-09	1.97E-10	4.66E-10
G1AM3	427	80	3 μL	1.54E-09		9.62E-11	
G1BM1	450	80	3 μL	1.29E-08		7.64E-10	
G1BM2	360	80	3 μL	4.80E-07	1.64E-07	3.56E-08	1.21E-08
G1BM3	540	80	3 μL	0.00E+00		0.00E+00	
S1AF1	50	80	3 μL	2.62E-05		1.40E-05	
S1AF2	50	80	3 μL	7.66E-06	1.40E-05	4.09E-06	7.49E-06
S1AF3	50	80	3 μL	8.26E-06		4.41E-06	
S1AM1	50	80	3 μL	0.00E+00		0.00E+00	
S1AM2	50	80	3 μL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
S1AM3	60	80	3 μL	N/A		N/A	
S1AR1	50	80	3 μL	1.03E-04		5.49E-05	
S1AR2	50	80	3 μL	0.00E+00	3.49E-05	0.00E+00	1.86E-05
S1AR3	50	80	3 μL	1.75E-06		9.33E-07	
S2BF1	50	80	3 μL	0.00E+00		0.00E+00	
S2BF2	60	80	3 μL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
S2BF3	50	80	3 μL	0.00E+00		0.00E+00	
S2BM1	60	80	3 μL	0.00E+00		0.00E+00	
S2BM2	50	80	3 μL	0.00E+00	2.37E-07	0.00E+00	1.05E-07
S2BM3	60	80	3 μL	7.10E-07		3.16E-07	
S2BR1	50	80	3 μL	0.00E+00		0.00E+00	
S2BR2	50	80	3 μL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
S2BR3	50	80	3 μL	0.00E+00		0.00E+00	
G3AF1	200	80	3 μL	0.00E+00		0.00E+00	
G3AF2	100	80	3 μL	1.46E-06	4.87E-07	3.89E-07	1.30E-07
G3AF3	200	80	3 μL	0.00E+00		0.00E+00	
G3AM1	200	80	3 μL	2.95E-07		3.93E-08	
G3AM2	200	80	3 μL	9.30E-07	4.08E-07	1.24E-07	5.44E-08

المنارة للاستشارات
www.manaraa.com

Table 3.7 (continued)

G3AM3	200	80	3 µL	0.00E+00		0.00E+00	
G3AR1	100	80	3 µL	1.57E-06		4.19E-07	
G3AR2	100	80	3 µL	2.77E-06	6.45E-06	7.39E-07	1.72E-06
G3AR3	100	80	3 µL	1.50E-05		4.00E-06	
G4BF1	100	80	3 µL	0.00E+00		0.00E+00	
G4BF2	200	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G4BF3	200	80	3 µL	0.00E+00		0.00E+00	
G4BM1	300	80	3 µL	0.00E+00		0.00E+00	
G4BM2	200	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G4BM3	200	80	3 µL	0.00E+00		0.00E+00	
G4BR1	100	80	3 µL	0.00E+00		0.00E+00	
G4BR2	100	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G4BR3	100	80	3 µL	0.00E+00		0.00E+00	

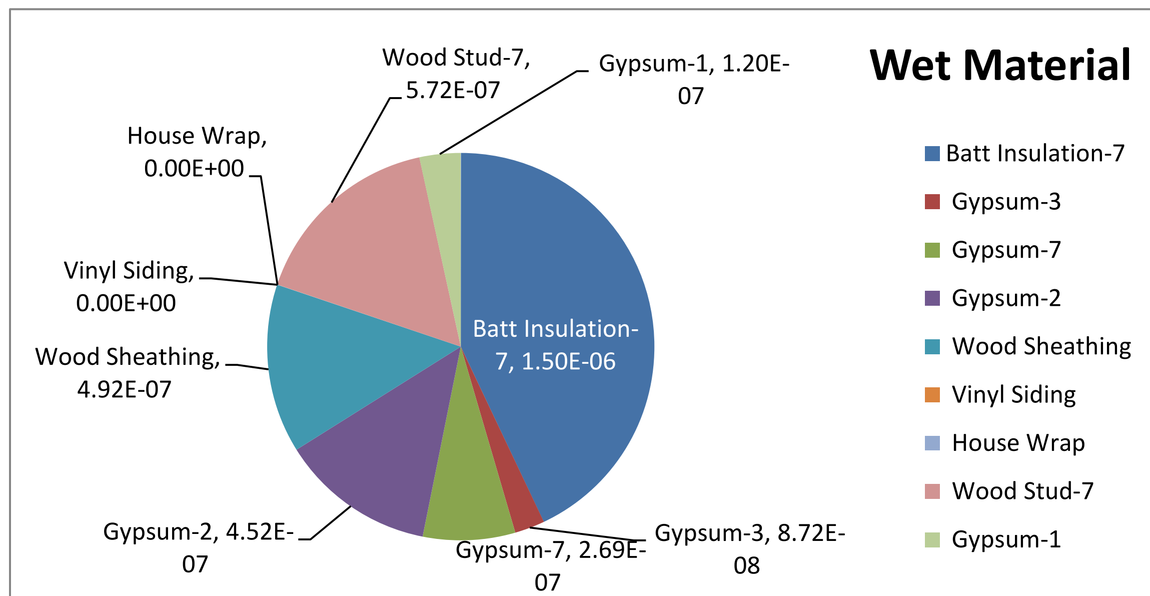

Figure 3.12 The distribution of *Aspergillus niger*, based on averaged DNA concentrations, on the different wet building materials. Samples with a 7 came from the same wall unit.

Overall concentrations of *Aspergillus niger* were low compared to the other mold species and ranged from a high concentration of 1.50E^{-06} on the fiberglass batt insulation

المنارة للاستشارات
www.manaraa.com

to non-detectable on the vinyl siding and house wrap. The concentration of *A.niger* on the batt insulation was statistically greater than on any of the other wall material (Table 3.8).

Table 3.8 Statistical (ANOVA) anaylsis of the significant difference of dry building materials. (Means with the same letter are not significantly different).

ANOVA Statistical Difference				
Tukey Grouping		**Mean**	**N**	**Material**
	A	1.49967E-6	18	Batt Insulation
B	A	4.74218E-7	17	Gypsum-2
B	A	2.79375E-7	17	Gypsum-7
B	A	1.90769E-7	18	Wood stud
B	A	1.23073E-7	12	Wood sheathing
B	A	1.20401E-7	18	Gypsum-1
B		7.79681E-8	17	Gypsum-3
B		0	6	Vinyl siding
B		0	6	House wrap

The distribution of *Aspergillus niger* on the individual wall materials are shown in Figures 3.13-3.22.

المنارة للاستشارات
www.manaraa.com

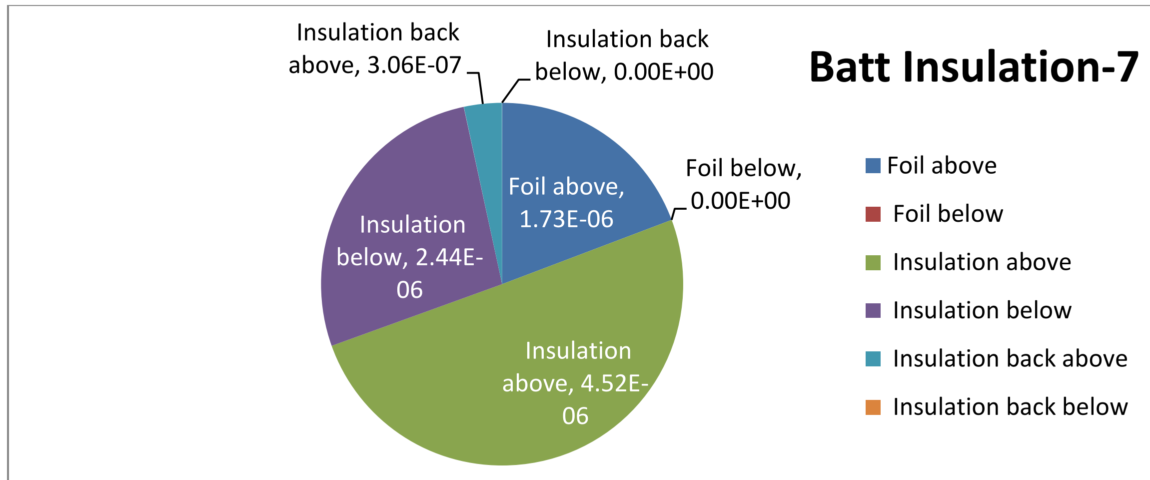

Figure 3.13 The distribution of *Aspergillus niger*, based on DNA concentrations, on the different components of batt insulation-7.

Aspergillus niger was found in the highest concentration of $4.52E^{-06}$ on the insulation itself (green) above the water line compared to the insulation below the water line ($2.44E^{-06}$) and the foil above the water line ($1.73E^{-06}$). It is interesting to note that *Aspergillus niger* occurred on the back side of the insulation above the water line but wasn't found on the back side of the insulation below water line. The species was also found on the foil above the water line yet not on the foil below the water line. With the exception of the insulation below the water line, *A. niger* was found primarily above the water line.

المنارة للاستشارات
www.manaraa.com

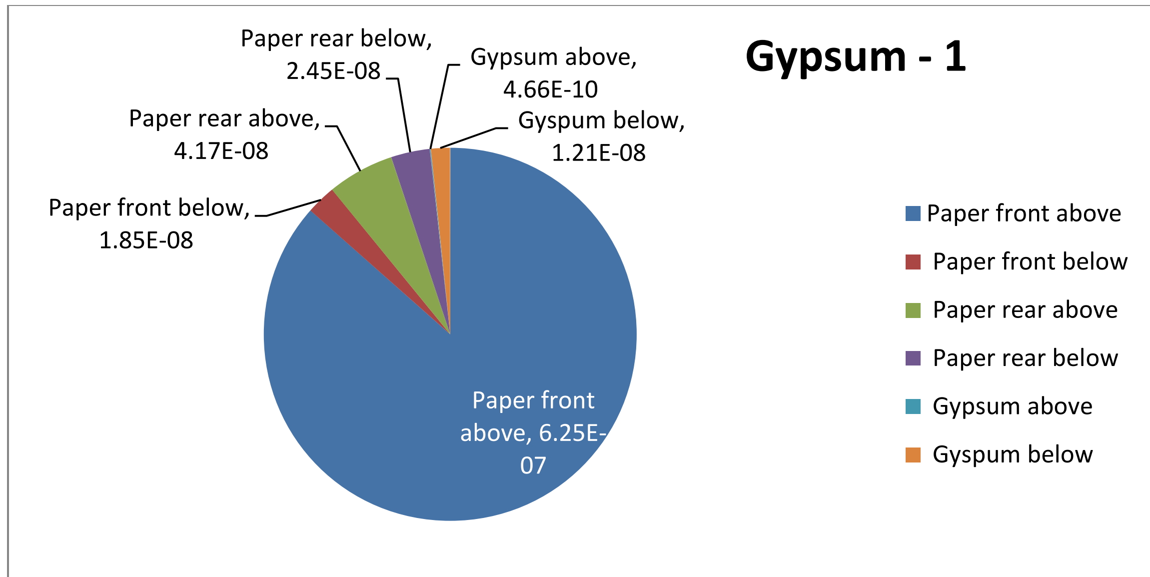

Figure 3.14 The distribution of *Aspergillus niger*, based on DNA concentrations, on the different components of gypsum-1.

Figure 3.14 showed the presence of *Aspergillus niger* in low concentrations on all locations of the gypsum-1 wallboard. The highest concentration was on the paper front above the water line ($6.25E^{-07}$) while all other components were found at 10^{-8} or less concentrations.

www.manaraa.com

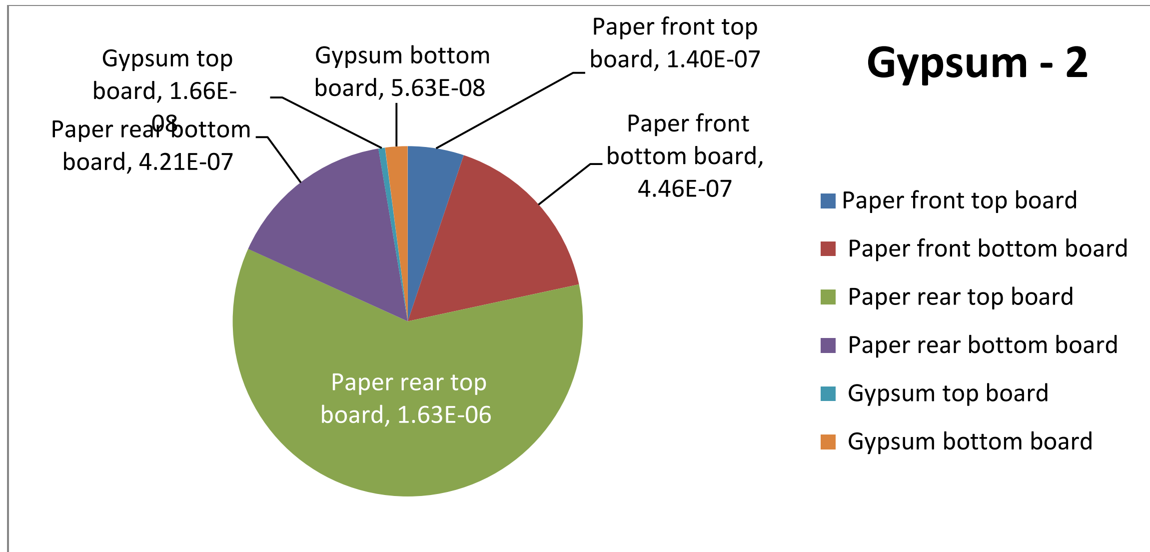

Figure 3.15 The distribution of *Aspergillus niger*, based on DNA concentrations, on the different components of gypsum-2.

Gypsum -2 shown in Figure 3.15 found *Aspergillus niger* present in moderately low concentrations on the paper located on the rear and top of the board ($1.63E^{-06}$). This was followed by lower concentrations (10^{-7}) on the other paper components, while the gypsum itself contained *A. niger* concentration of 10^{-8}.

المنارة للاستشارات
www.manaraa.com

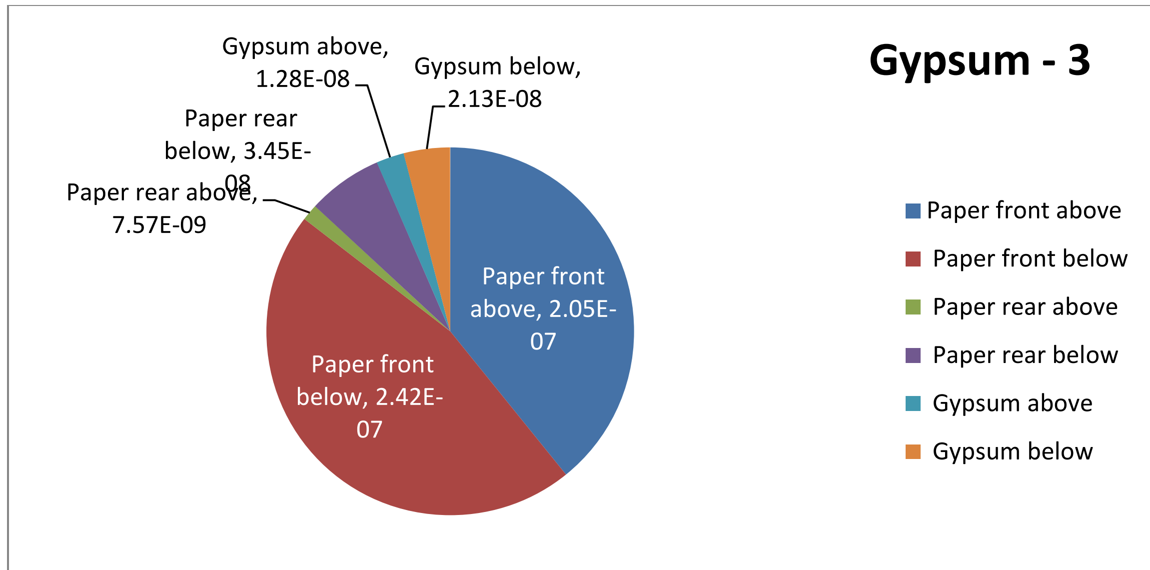

Figure 3.16 The distribution of *Aspergillus niger*, based on DNA concentrations, on the different components of gypsum-3.

For gypsum-3 (Figure 3.16) *Aspergillus niger* was found at low concentrations on the paper front both below ($2.42E^{-07}$) and above ($2.05E^{-07}$) the water line. *Aspergillus niger* was also found on the other gypsum components including the rear paper both above and below the water line (averaged $2.10E^{-08}$) and gypsum both above and below the water line (averaged $1.71E^{-08}$).

www.manaraa.com

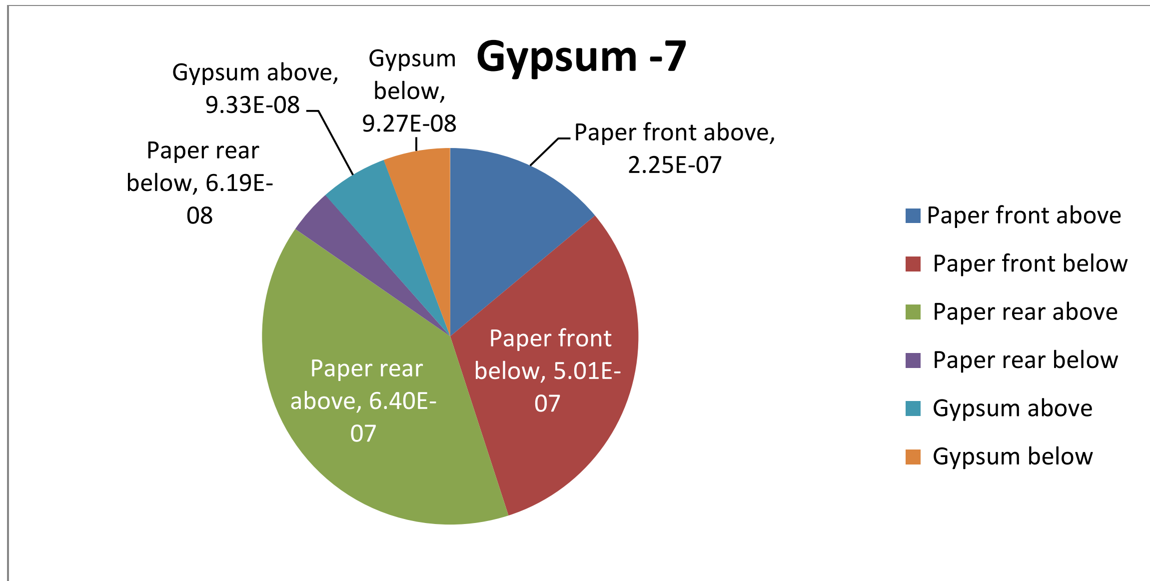

Figure 3.17 The distribution of *Aspergillus niger*, based on DNA concentrations, on the different components of the gypsum-7.

Figure 3.17 shows that *Aspergillus niger* was present on all of the wall components of gypsum-7 including paper and gypsum both above and below the water line. However, all concentrations of *A. niger* were low ranging from a high of $6.40E^{-07}$ on paper rear above the water line to a low of ($6.19E^{-08}$) on the rear paper below the water line.

المنارة للاستشارات
www.manaraa.com

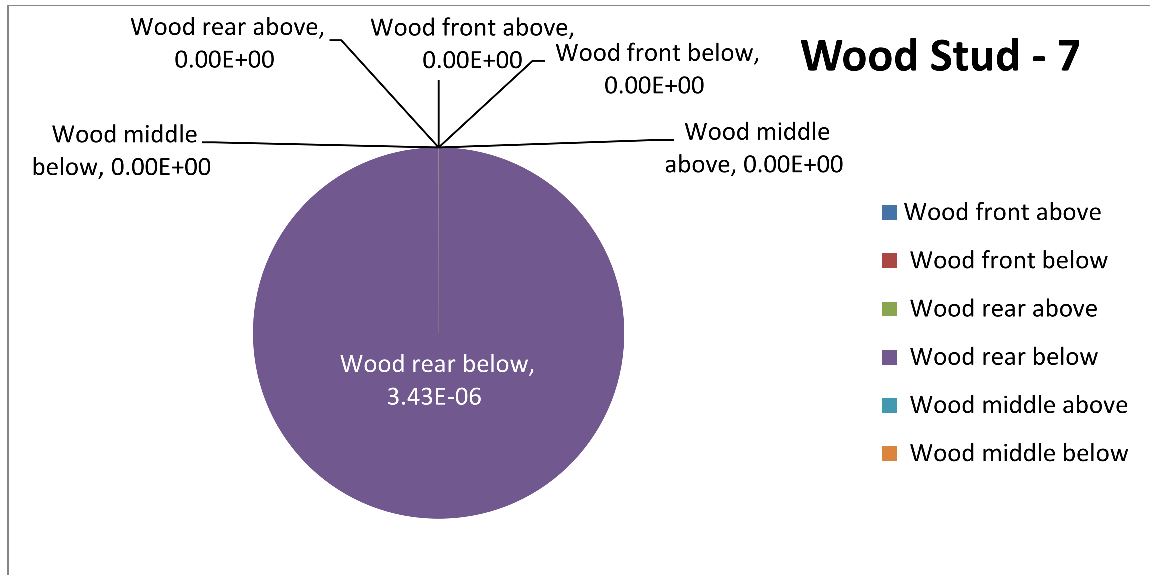

Figure 3.18 The distribution of *Aspergillus niger*, based on DNA concentrations, on the different components of the wood stud-7.

Figure 3.18 shows that *Aspergillus niger* was only found on the rear side of the 2x4 wood stud-7 below the water line ($3.43E^{-06}$). All other components of the wood stud contained no *Aspergillus niger*. Even though *A.niger* was only found in one location of the wood stud, this concentration is actually the highest concentration of *A. niger* detected for any individual wet building material component.

المنارة للاستشارات
www.manaraa.com

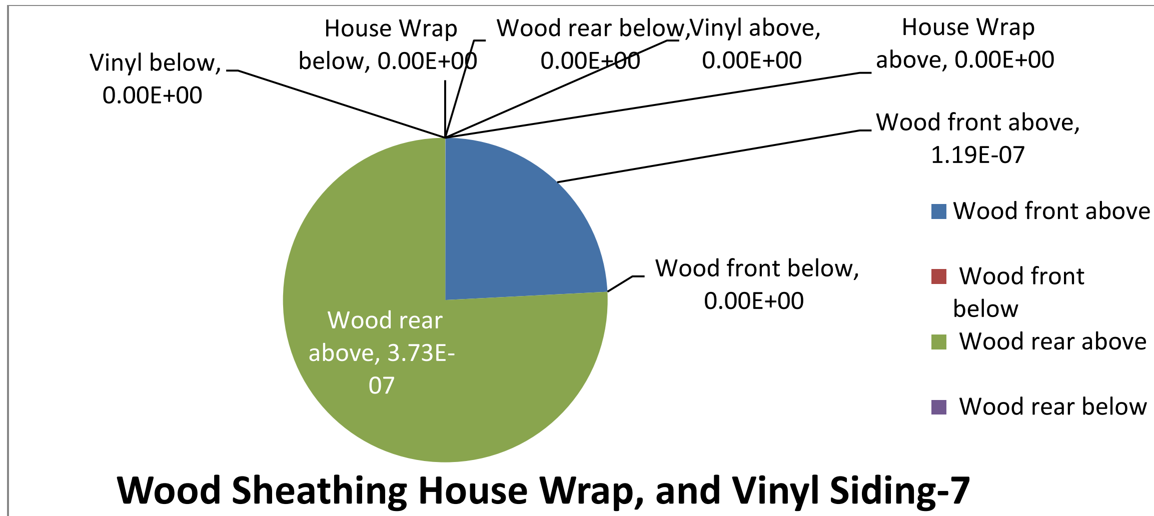

Figure 3.19 The distribution of *Aspergillus niger*, based on DNA concentrations, on the different components of the wet wood sheathing, plus the house wrap, and vinyl siding-7.

Aspergillus niger was only found on the wood sheathing at low concentrations in Figure 3.19. The wood sheathing on the rear side below the water line contained a concentration of 3.73E^{-07} while the wood sheathing on the front above the water line contained a concentration of 1.19E^{-07}. *A. niger* was not detected on any other component of the wood sheathing nor on the vinyl siding or house wrap. Statistics were performed on both above and below the water line for *A. niger* and these results are given in Table 3.9.

Table 3.9 Statistical analysis on *Aspergillus niger* both above and below the water line on wet materials.

State	Water line	Samples	Mean	Std Dev
wet	above	57	4.75E-07	1.54E-06
wet	below	57	2.42E-07	7.22E-07

المنارة للاستشارات
www.manaraa.com

Table 3.9 shows that the average concentration of *Aspergillus niger* was higher above the water line than below the water line. The dry samples were examined for the presence of *Aspergillus niger* on the wood stud and gypsum wallboard and is shown in Figure 3.20.

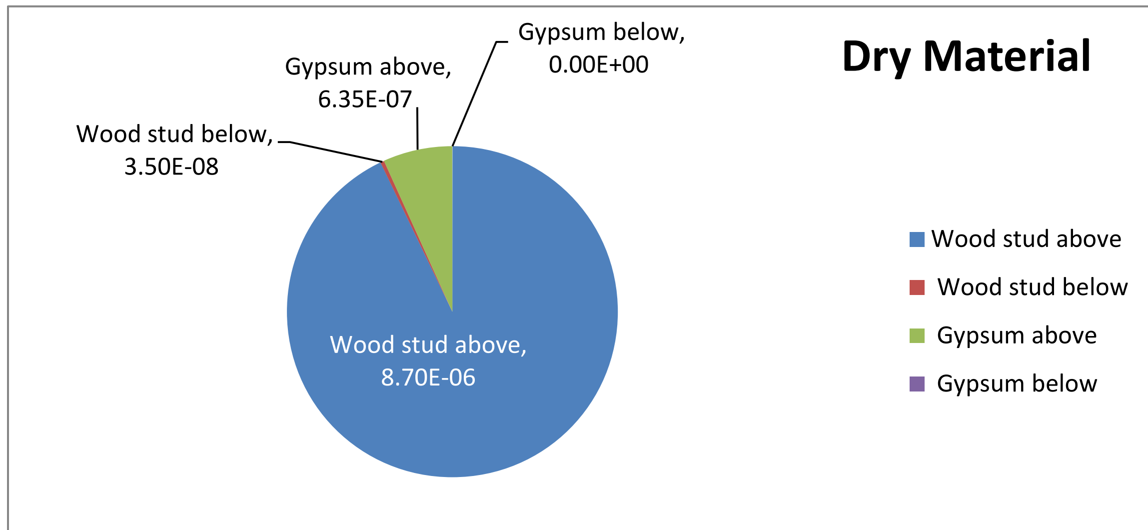

Figure 3.20 The distribution of *Aspergillus niger*, based on DNA concentrations, on the different components of dry building materials.

Figure 3.20 shows that *Aspergillus niger* was present on all dry materials except on the gypsum wallboard below the water line. The wood stud above the water line contained the highest concentration of *A. niger* ($8.70E^{-06}$) while the gypsum wallboard above the water line ($6.35E^{-07}$) and wood stud below ($3.50E^{-08}$) contained much lower concentrations of *Aspergillus niger*. There were no statistical differences found in *A. niger* concentrations among the different dry materials (Table 3.10).

للاستشارات المنارة
www.manaraa.com

Table 3.10 Statistical (ANOVA) anaylsis of the significant difference of dry building materials. (Means with the same letter are not significantly different).

ANOVA Statistical Difference			
Tukey Grouping	**Mean**	**N**	**Material**
A	4.62625E-6	17	Wood stud
A	3.17222E-7	18	Gypsum

The presence of *Aspergillus niger* was examined on the individual dry materials components and these are presented in Figures 3.21-3.22

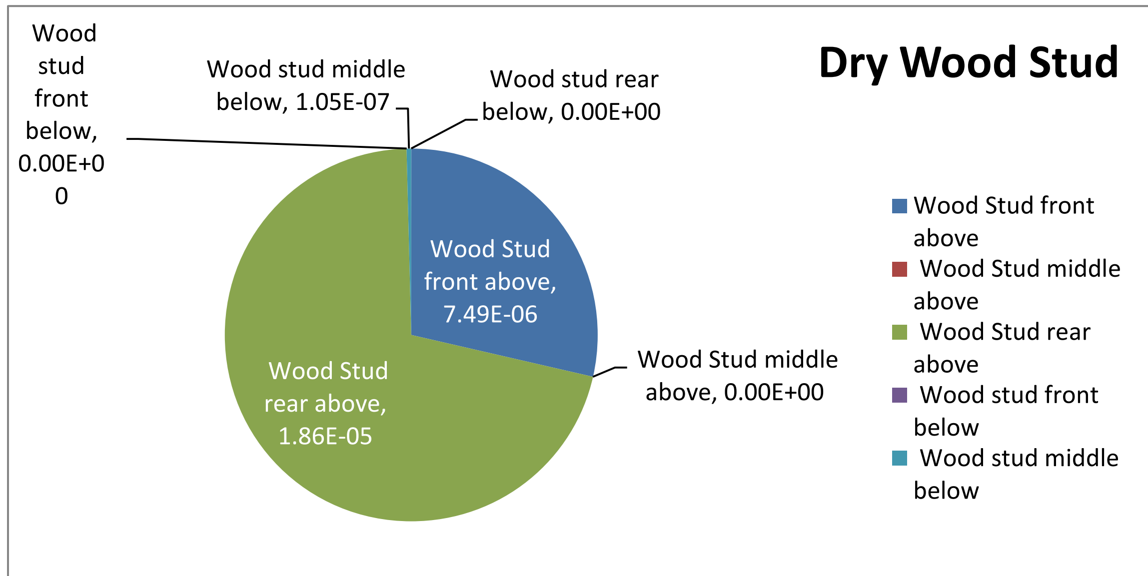

Figure 3.21 The distribution of *Aspergillus niger*, based on DNA concentrations, on the different components of the dry wood stud.

Figure 3.21 shows that *Aspergillus niger* was detected in concentrations of $1.86E^{-05}$ on the rear section of the wood stud above the water line while only $7.49E^{-06}$ was found on the front section of the wood stud above the water line. The middle section of the 2x4

المنارة للاستشارات
www.manaraa.com

wood stud below the water line supported a low concentration of *A. niger* ($1.05E^{-07}$). *A. niger* was not detected on any of the other components of the dry wood stud.

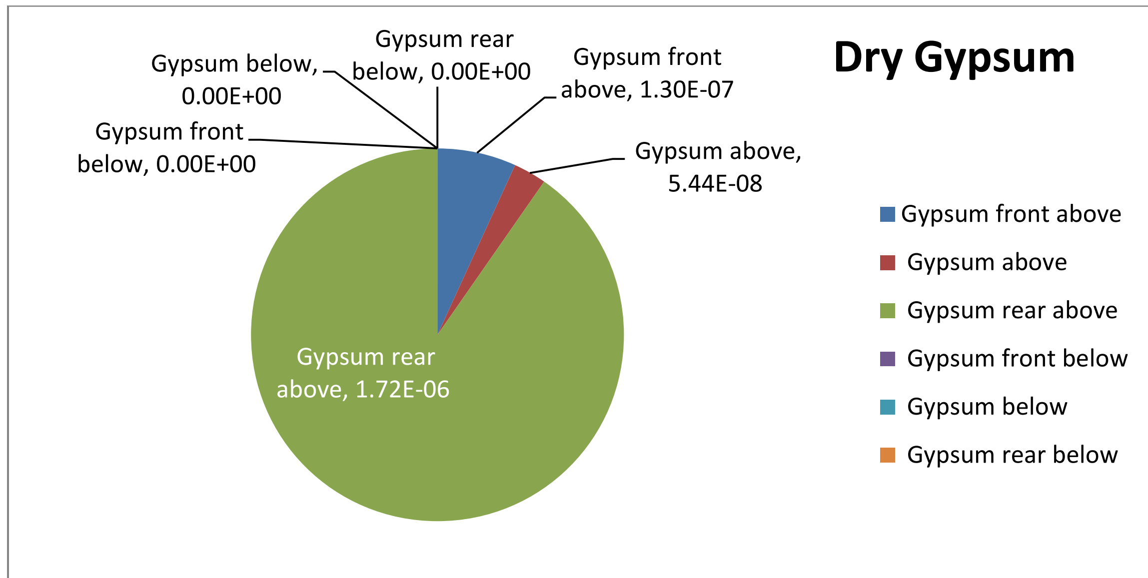

Figure 3.22 The distribution of *Aspergillus niger*, based on DNA concentrations, on the different components of the dry gypsum.

The distribution of *Aspergillus niger* on the dry gypsum board was predominantly located on the rear paper above the water line ($1.72E^{-06}$) in Figure 3.22. The other gypsum material samples that conained low levels of *Aspergillus niger* was gypsum above the water line ($5.44E^{-08}$) and the paper front above the water line ($1.30E^{-07}$).

Overall the concentrations of *A. niger* were quite low compared to the other mold species. On the wet materials, the batt insulation contained the greatest average of *A. niger* indicating that in total, there was more *A. niger* on the batt compared to the other building materials. However, the highest concentration of *A. niger* on a single component of a wet building material was found on the rear of the wood stud. When comparing wet

المنارة للاستشارات
www.manaraa.com

versus dry materials, this species was found in the greatest concentration on the dry wood stud. The dry wood stud supported both the highest average and the highest individual concentration of *A. niger*. The dry gypsum also supported moderate levels of this species. In addition *A. niger* was two times more likely to be above the water line versus below the water line. This implies that *A. niger* does not survive well in very wet conditions and is more likely to thrive in lower moisture environments.

Aspergillus terreus

Aspergillus terreus occurs worldwide, especially in soil. It is considered uncommon on building materials; however, it has been found in floor, carpet, and mattress dust (Flannigan et al. 2011). *Aspergillus terreus* is in the phylum Ascomycota and is a filamentous fungus that consists of a dense velvety felt of yellow brown conidiophores mixed with aerial hyphae bearing conidiophores (Samson et al. 2007). *Aspergillus terreus* is considered an important human pathogen which can cause pulmonary aspergillosis. The DNA concentrations of *Aspergillus terreus* were determined using real-time PCR of each wall construction material and converted to the amount of fungal DNA in nannograms per weight of the material sample (Table 3.11).

المنارة للاستشارات
www.manaraa.com

Table 3.11 Real-time PCR results of *Aspergillus terreus* DNA in nannograms per weight of material

Sample Code	Material Weight (mg)	Extraction volume	Amount µL used	Real Time (ng)	Average Reps	(ng)DNA/(mg) Material	Average DNA/Material
B1AP1	100	80	3 µL	7.45E-03		1.99E-03	
B1AP2	100	80	3 µL	2.39E-03	3.32E-03	6.37E-04	8.84E-04
B1AP3	100	80	3 µL	1.05E-04		2.80E-05	
B1BP1	140	80	3 µL	0.00E+00		0.00E+00	
B1BP2	140	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
B1BP3	140	80	3 µL	0.00E+00		0.00E+00	
B1AN1	50	80	3 µL	3.20E-03		1.71E-03	
B1AN2	50	80	3 µL	3.80E-04	1.32E-03	2.03E-04	7.01E-04
B1AN3	50	80	3 µL	3.65E-04		1.95E-04	
B1BN1	100	80	3 µL	2.40E-04		6.40E-05	
B1BN2	100	80	3 µL	2.44E-04	2.05E-04	6.51E-05	5.47E-05
B1BN3	100	80	3 µL	1.31E-04		3.49E-05	
B1AF1	50	80	3 µL	5.76E-04		3.07E-04	
B1AF2	50	80	3 µL	5.56E-03	2.09E-03	2.97E-03	1.12E-03
B1AF3	50	80	3 µL	1.36E-04		7.25E-05	
B1BF1	100	80	3 µL	0.00E+00		0.00E+00	
B1BF2	100	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
B1BF3	100	80	3 µL	0.00E+00		0.00E+00	
G3AS1	200	80	3 µL	2.07E-05		2.76E-06	
G3AS2	200	80	3 µL	9.94E-05	4.08E-05	1.33E-05	5.44E-06
G3AS3	200	80	3 µL	2.38E-06		3.17E-07	
G3BS1	250	80	3 µL	0.00E+00		0.00E+00	
G3BS3	250	80	3 µL	3.22E-05		3.43E-06	
G3AR1	250	80	3 µL	6.45E-06		6.88E-07	
G3AR2	250	80	3 µL	0.00E+00	3.30E-06	0.00E+00	3.52E-07
G3AR3	250	80	3 µL	3.46E-06		3.69E-07	
G3BR1	300	80	3 µL	1.67E-04		1.48E-05	
G3BR2	300	80	3 µL	0.00E+00	5.57E-05	0.00E+00	4.95E-06
G3BR3	300	80	3 µL	0.00E+00		0.00E+00	
G3AM1	500	80	3 µL	0.00E+00		0.00E+00	
G3AM2	500	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G3AM3	500	80	3 µL	0.00E+00		0.00E+00	
G3BM1	500	80	3 µL	0.00E+00		0.00E+00	
G3BM2	500	80	3 µL	0.00E+00	1.42E-06	0.00E+00	7.59E-08

www.manaraa.com

Table 3.11 (continued)

G3BM3	500	80	3 μL	4.27E-06		2.28E-07	
G4AS1	200	80	3 μL	5.71E-05		7.61E-06	
G4AS2	230	80	3 μL	0.00E+00	4.62E-05	0.00E+00	6.16E-06
G4AS3	200	80	3 μL	8.15E-05		1.09E-05	
G4BS1	210	80	3 μL	4.77E-05		6.06E-06	
G4BS2	200	80	3 μL	3.98E-04	1.93E-04	5.31E-05	2.57E-05
G4BS3	200	80	3 μL	1.34E-04		1.79E-05	
G4AR1	100	80	3 μL	1.00E-05		2.67E-06	
G4AR2	100	80	3 μL	3.29E-05	1.43E-05	8.77E-06	3.81E-06
G4AR3	100	80	3 μL	0.00E+00		0.00E+00	
G4BR1	200	80	3 μL	1.80E-05		2.40E-06	
G4BR2	200	80	3 μL	2.71E-06	1.22E-05	3.61E-07	1.62E-06
G4BR3	200	80	3 μL	1.58E-05		2.11E-06	
G4AM1	300	80	3 μL	9.63E-05		8.56E-06	
G4AM2	300	80	3 μL	0.00E+00	3.34E-05	0.00E+00	2.96E-06
G4AM3	320	80	3 μL	3.91E-06		3.26E-07	
G4BM1	500	80	3 μL	0.00E+00		0.00E+00	
G4BM2	520	80	3 μL	6.65E-05	2.22E-05	3.41E-06	1.14E-06
G4BM3	500	80	3 μL	0.00E+00		0.00E+00	
G2TS1	150	80	3 μL	0.00E+00		0.00E+00	
G2TS2	160	80	3 μL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G2TS3	150	80	3 μL	0.00E+00		0.00E+00	
G2BS1	160	80	3 μL	0.00E+00		0.00E+00	
G2BS2	150	80	3 μL	5.96E-05	3.59E-05	1.06E-05	6.38E-06
G2BS3	150	80	3 μL	4.81E-05		8.55E-06	
G2TR1	100	80	3 μL	0.00E+00		0.00E+00	
G2TR2	100	80	3 μL	0.00E+00	1.67E-03	0.00E+00	4.46E-04
G2TR3	100	80	3 μL	5.02E-03		1.34E-03	
G2BR1	100	80	3 μL	1.84E-05		4.91E-06	
G2BR2	80	80	3 μL	3.79E-05	3.16E-05	1.26E-05	1.01E-05
G2BR3	80	80	3 μL	3.85E-05		1.28E-05	
G2TM1	3150	80	3 μL	0.00E+00		0.00E+00	
G2TM2	100	80	3 μL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G2TM3	200	80	3 μL	0.00E+00		0.00E+00	
G2BM1	170	80	3 μL	0.00E+00		0.00E+00	
G2BM2	280	80	3 μL	4.08E-06	3.91E-06	3.89E-07	4.70E-07
G2BM3	200	80	3 μL	7.66E-06		1.02E-06	
W7AS1	100	80	3 μL	4.54E-07		1.21E-07	

المنارة للاستشارات
www.manaraa.com

Table 3.11 (continued)

W7AS2	100	80	3 µL	3.36E-07	9.60E-07	8.96E-08	2.03E-07
W7AS3	140	80	3 µL	2.09E-06		3.98E-07	
W7BS1	50	80	3 µL	1.46E-04		7.79E-05	
W7BS2	50	80	3 µL	1.27E-04	1.40E-04	6.77E-05	8.10E-05
W7BS3	40	80	3 µL	1.46E-04		9.73E-05	
W7AR1	40	80	3 µL	4.23E-06		2.82E-06	
W7AR2	80	80	3 µL	1.92E-06	2.05E-06	6.40E-07	1.15E-06
W7AR3	80	80	3 µL	0.00E+00		0.00E+00	
W7BR1	50	80	3 µL	3.49E-04		1.86E-04	
W7BR2	60	80	3 µL	4.98E-04	3.77E-04	2.21E-04	1.86E-04
W7BR3	50	80	3 µL	2.85E-04		1.52E-04	
I7A1	40	80	3 µL	2.97E-08		1.98E-08	
I7A2	40	80	3 µL	0.00E+00	1.37E-07	0.00E+00	9.10E-08
I7A3	40	80	3 µL	3.80E-07		2.53E-07	
I7B1	60	80	3 µL	8.65E-05		3.84E-05	
I7B2	50	80	3 µL	0.00E+00	2.88E-05	0.00E+00	1.28E-05
I7B3	50	80	3 µL	0.00E+00		0.00E+00	
V7A1	400	80	3 µL	0.00E+00		0.00E+00	
V7A2	400	80	3 µL	0.00E+00	3.80E-08	0.00E+00	2.53E-09
V7A3	400	80	3 µL	1.14E-07		7.60E-09	
V7B1	400	80	3 µL	0.00E+00		0.00E+00	
V7B2	400	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
V7B3	300	80	3 µL	0.00E+00		0.00E+00	
W4AS1	80	80	3 µL	1.50E-04		5.00E-05	
W4AS2	90	80	3 µL	7.40E-04	3.41E-04	2.19E-04	9.13E-05
W4AS3	760	80	3 µL	1.34E-04		4.70E-06	
W4BS1	900	80	3 µL	4.17E-05		1.24E-06	
W4BS2	76	80	3 µL	2.29E-04	9.28E-05	8.04E-05	2.77E-05
W4BS3	130	80	3 µL	7.69E-06		1.58E-06	
W4BR1	150	80	3 µL	0.00E+00		0.00E+00	
W4AR2	90	80	3 µL	0.00E+00	1.53E-06	0.00E+00	5.84E-07
W4AR3	70	80	3 µL	4.60E-06		1.75E-06	
W4BR1	40	80	3 µL	2.13E-05		1.42E-05	
W4BR2	65	80	3 µL	2.90E-05	2.85E-05	1.19E-05	1.50E-05
W4BR3	50	80	3 µL	3.52E-05		1.88E-05	
W4AM1	60	80	3 µL	0.00E+00		0.00E+00	
W4AM2	50	80	3 µL	2.70E-06	9.00E-07	1.44E-06	4.80E-07
W4AM3	40	80	3 µL	0.00E+00		0.00E+00	

المنارة للاستشارات
www.manaraa.com

Table 3.11 (continued)

W4BM1	53	80	3 μL	0.00E+00		0.00E+00	
W4BM2	50	80	3 μL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
W4BM3	66	80	3 μL	0.00E+00		0.00E+00	
G1AS1	120	80	3 μL	1.24E-05		2.76E-06	
G1AS2	170	80	3 μL	0.00E+00	4.13E-06	0.00E+00	9.19E-07
G1AS3	200	80	3 μL	0.00E+00		0.00E+00	
G1BS1	200	80	3 μL	0.00E+00		0.00E+00	
G1BS2	300	80	3 μL	0.00E+00	1.21E-05	0.00E+00	6.47E-07
G1BS3	500	80	3 μL	3.64E-05		1.94E-06	
G1AR1	62	80	3 μL	0.00E+00		0.00E+00	
G1AR2	75	80	3 μL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G1AR3	100	80	3 μL	0.00E+00		0.00E+00	
G1BR1	97	80	3 μL	0.00E+00		0.00E+00	
G1BR2	100	80	3 μL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G1BR3	150	80	3 μL	0.00E+00		0.00E+00	
G1AM1	500	80	3 μL	0.00E+00		0.00E+00	
G1AM2	430	80	3 μL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G1AM3	427	80	3 μL	0.00E+00		0.00E+00	
G1BM1	450	80	3 μL	0.00E+00		0.00E+00	
G1BM2	360	80	3 μL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G1BM3	540	80	3 μL	0.00E+00		0.00E+00	
S1AF1	50	80	3 μL	2.71E-04		1.45E-04	
S1AF2	50	80	3 μL	6.03E-05	2.28E-04	3.22E-05	1.22E-04
S1AF3	50	80	3 μL	3.53E-04		1.88E-04	
S1AM1	50	80	3 μL	0.00E+00		0.00E+00	
S1AM2	50	80	3 μL	0.00E+00	3.47E-06	0.00E+00	1.54E-06
S1AM3	60	80	3 μL	1.04E-05		4.62E-06	
S1AR1	50	80	3 μL	3.56E-03		1.90E-03	
S1AR2	50	80	3 μL	3.30E-04	1.37E-03	1.76E-04	7.30E-04
S1AR3	50	80	3 μL	2.15E-04		1.15E-04	
S2BF1	50	80	3 μL	1.62E-04		8.64E-05	
S2BF2	60	80	3 μL	4.18E-04	1.93E-04	1.86E-04	9.07E-05
S2BF3	50	80	3 μL	0.00E+00		0.00E+00	
S2BM1	60	80	3 μL	6.59E-04		2.93E-04	
S2BM2	50	80	3 μL	1.24E-02	4.40E-03	6.61E-03	2.32E-03
S2BM3	60	80	3 μL	1.48E-04		6.58E-05	
S2BR1	50	80	3 μL	0.00E+00		0.00E+00	
S2BR2	50	80	3 μL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
S2BR3	50	80	3 μL	0.00E+00		0.00E+00	

المنارة للاستشارات
www.manaraa.com

Table 3.11 (continued)

G3AF1	200	80	3 µL	0.00E+00		0.00E+00	
G3AF2	100	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G3AF3	200	80	3 µL	0.00E+00		0.00E+00	
G3AM1	200	80	3 µL	1.70E-05		2.27E-06	
G3AM2	200	80	3 µL	1.59E-05	1.65E-05	2.12E-06	2.20E-06
G3AM3	200	80	3 µL	1.65E-05		2.20E-06	
G3AR1	100	80	3 µL	3.47E-06		9.25E-07	
G3AR2	100	80	3 µL	3.82E-05	2.67E-05	1.02E-05	7.13E-06
G3AR3	100	80	3 µL	3.85E-05		1.03E-05	
G4BF1	100	80	3 µL	0.00E+00		0.00E+00	
G4BF2	200	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G4BF3	200	80	3 µL	0.00E+00		0.00E+00	
G4BM1	300	80	3 µL	2.72E-05		2.42E-06	
G4BM2	200	80	3 µL	8.69E-06	1.20E-05	1.16E-06	1.19E-06
G4BM3	200	80	3 µL	0.00E+00		0.00E+00	
G4BR1	100	80	3 µL	7.25E-05		1.93E-05	
G4BR2	100	80	3 µL	5.99E-05	4.41E-05	1.60E-05	1.18E-05
G4BR3	100	80	3 µL	0.00E+00		0.00E+00	

The wet building materials were analyzed for *Aspergillus terreus* on each type of wet material and this data are shown in Figure 3.23.

المنارة للاستشارات
www.manaraa.com

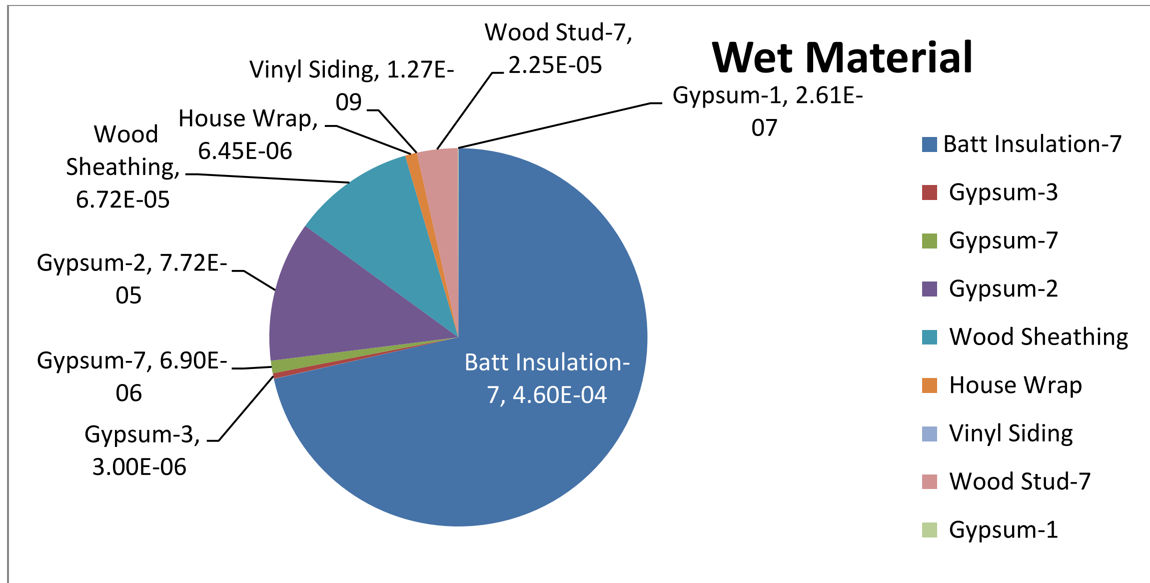

Figure 3.23 The distribution of *Aspergillus terreus*, based on averaged DNA concentrations, on the different wet building materials. Samples with a 7 came from the same wall unit.

The wet building material averages in Figure 3.23 show the presence of *Aspergillus terreus* on all materials. The fiberglass batt insulation contained the highest average concentration at $4.60E^{-04}$, followed by gypsum-2 ($7.72E^{-05}$), wood sheathing ($6.72E^{-05}$) and the wood stud ($2.25E^{-05}$). The vinyl siding contained extremely low concentrations of *A. terreus*. The concentration of *A. terreus* on the batt insulation was statistically greater than on any of the other wall materials (Table 3.12).

المنارة للاستشارات
www.manaraa.com

Table 3.12 Statistical (ANOVA) anaylsis of the significant difference of dry building materials. (Means with the same letter are not significantly different).

ANOVA Statistical Difference				
Tukey Grouping		**Mean**	**N**	**Material**
	A	0.0004592	18	Batt Insulation
B	A	0.0000772	18	Gypsum-2
B	A	0.0000672	12	Wood sheathing
B	A	0.0000225	18	Wood stud
B		0.0000069	18	Gypsum-7
B		0.0000065	6	House wrap
B		0.0000030	18	Gypsum-3
B		0.0000003	18	Gypsum-1
B		0.0000000	6	Vinyl siding

The distributions of *Aspergillus terreus* on individual components of each material are given in Figures 3.24- 3.30.

المنارة للاستشارات
www.manaraa.com

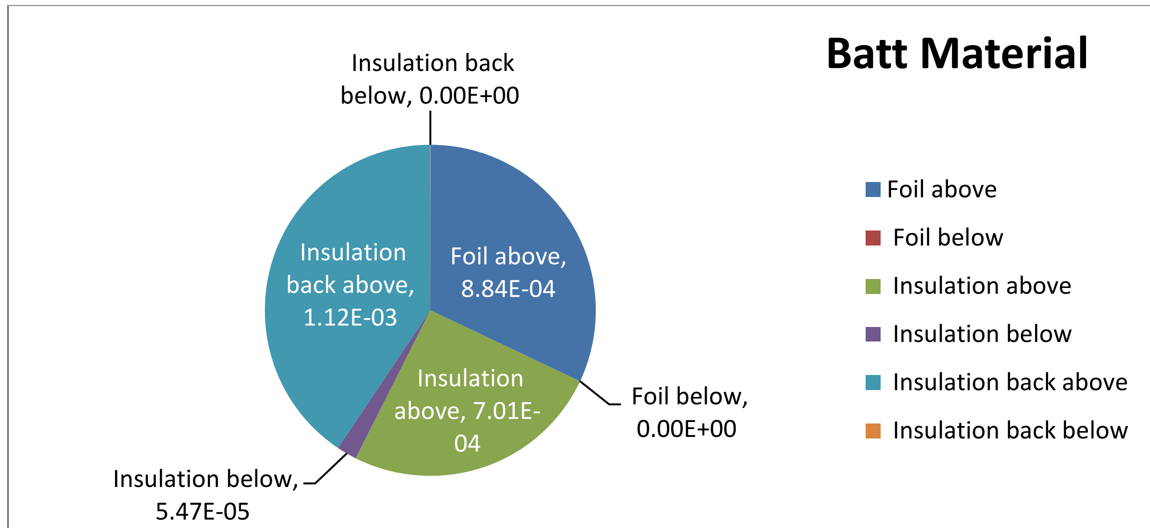

Figure 3.24 The distribution of *Aspergillus terreus*, based on DNA concentrations, on the different components of batt insulation-7.

The distribution of *Aspergillus terreus* in the batt insulation (Figure 3.24) indicates that this species was found in the highest concentration of $1.12E^{-03}$ above the water line on the back side of the insulation. It is interesting to note that *Aspergillus terreus* also occurred in moderate levels on the insulation above the water line ($7.01E^{-04}$) and the foil above the water line ($8.84E^{-04}$). Thus *A. terreus* appears to be primarily segregated to above the water line on the batt.

المنارة للاستشارات
www.manaraa.com

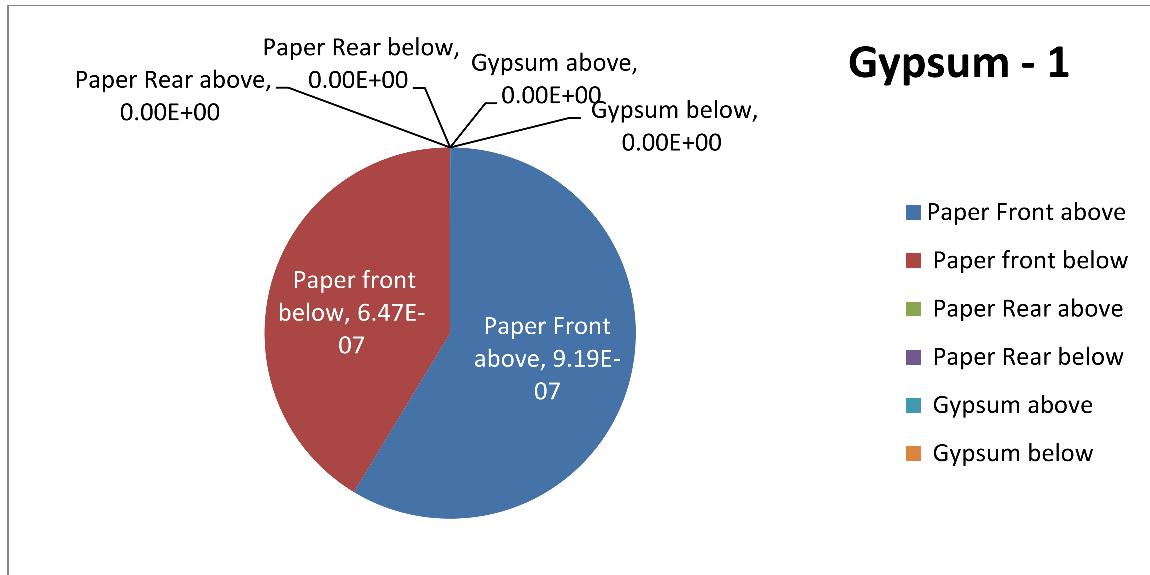

Figure 3.25 The distribution of *Aspergillus terreus*, based on averaged DNA concentrations, on the different components of wet gypsum-1.

Gypsum-1 in Figure 3.25 shows that *Aspergillus terreus* was found present in low concentrations in only two locations; on the paper front above the water line with a concentration of $9.19E^{-07}$ and below the water line with a concentration of $6.47E^{-07}$.

المنارة للاستشارات
www.manaraa.com

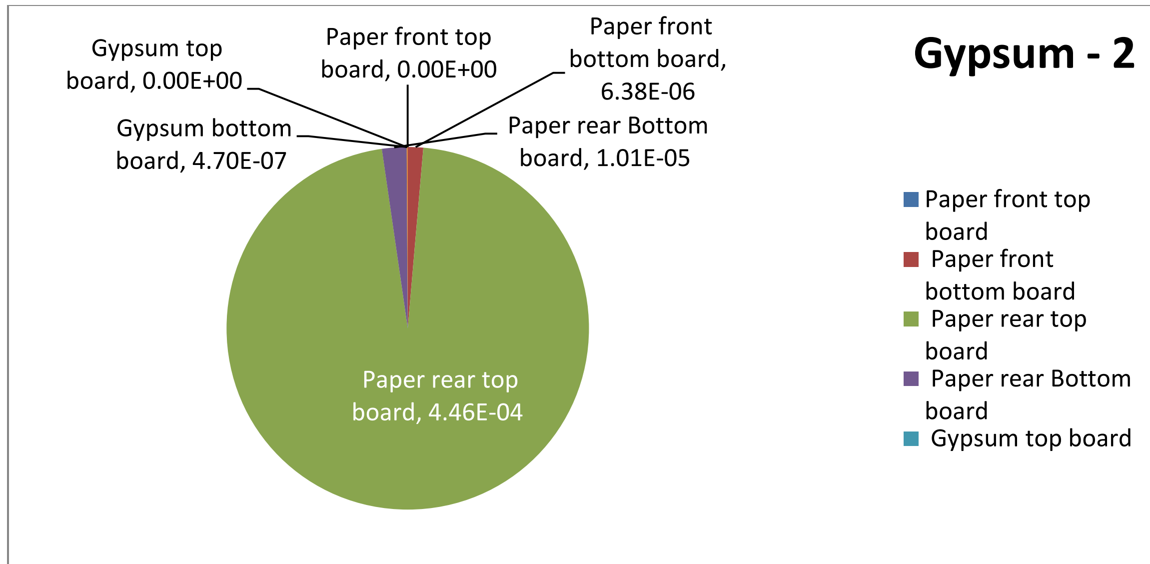

Figure 3.26 The distribution of *Aspergillus terreus*, based on DNA concentrations, on the different components of the wet Gypsum-2.

Gypsum -2 in Figure 3.26 shows that *Aspergillus terreus* was primarily located in moderate concentrations on the rear paper on the top of the board ($4.46E^{-04}$). The other components that contained *A. terreus* included the paper rear bottom of board ($1.01E^{-05}$) and the front paper on the bottom of the board ($6.38E^{-06}$). The gypsum itself contained low to no *A. terreus*.

المنارة للاستشارات
www.manaraa.com

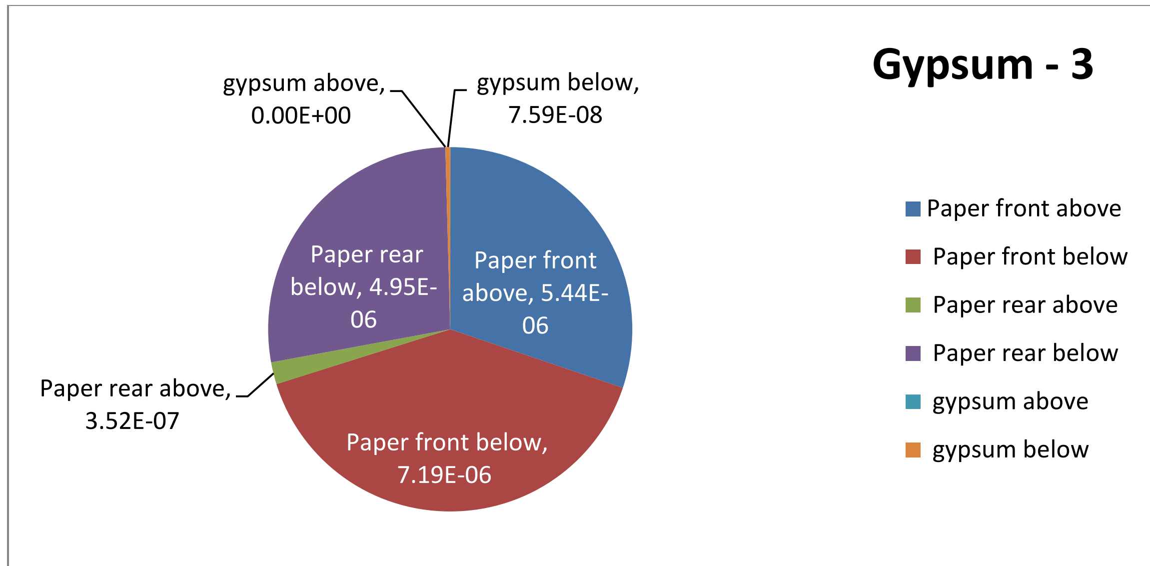

Figure 3.27 The distribution of *Aspergillus terreus*, based on DNA concentrations, on the different components of wet gypsum-3.

Aspergillus terreus was present on gypsum-3 (Figure 3.27) on all gypsum paper components but was not detected on the gypsum itself except in very low concentrations. The moderately low concentrations detected included the paper front below water line with a concentration of $7.19E^{-06}$, paper front above ($5.44E^{-06}$), and the paper rear below ($4.95E^{-06}$).

المنارة للاستشارات
www.manaraa.com

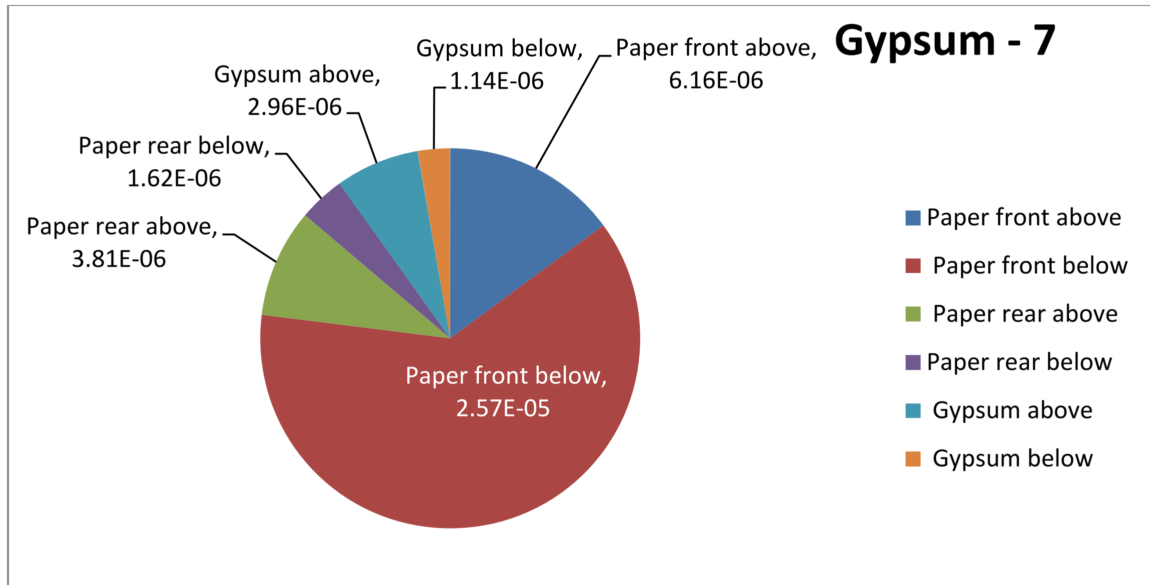

Figure 3.28 The distribution of *Aspergillus terreus*, based on DNA concentrations, on the different wet components of gypsum-7.

Figure 3.28 shows that *Aspergillus terreus* was present in the gypsum-7 board at a concentration of $2.57E^{-05}$ on the paper front below the water line as well as on the paper above ($6.16E^{-06}$). The paper rear above the water line ($3.81E^{-06}$) and the paper rear below the water line ($1.62E^{-06}$) all showed low concentrations of *Aspergillus terreus* present. It is worth noting that the gypsum above ($2.96E^{-06}$) had a higher concentration compared to gypsum below the water line ($1.14E^{-06}$). Also the gypsum itself contained moderately low levels of *A. terreus*.

المنارة للاستشارات
www.manaraa.com

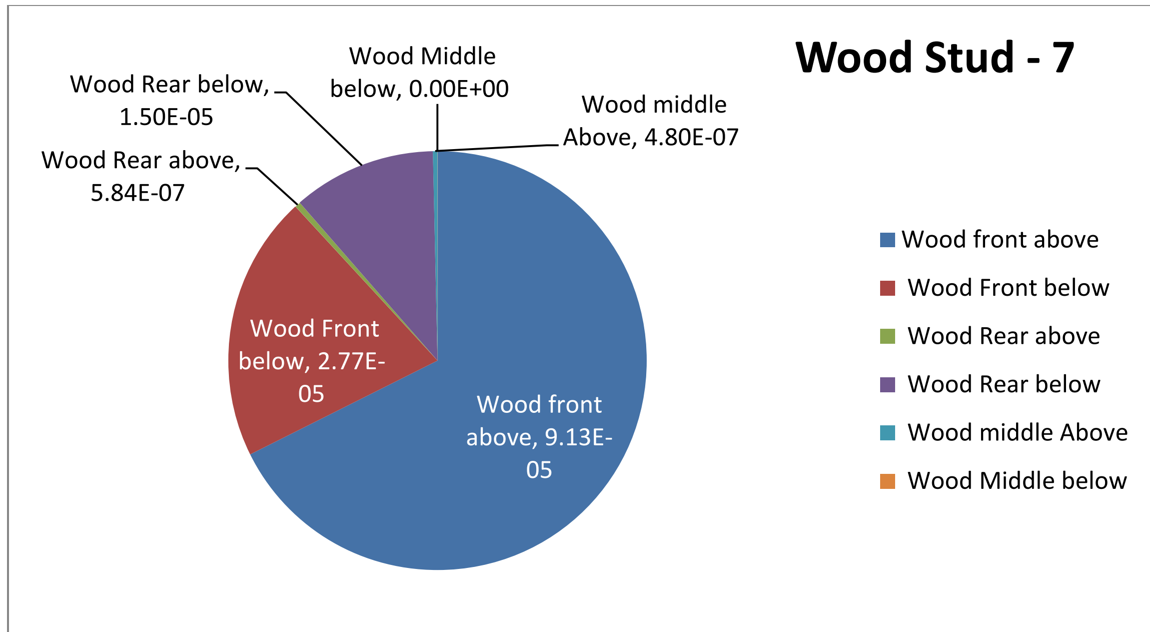

Figure 3.29 The distribution of *Aspergillus terreus*, based on DNA concentrations, on the different components of the wet wood stud-7.

Figure 3.29 shows that *Aspergillus terreus* was present in moderate concentrations on the front section of the 2x4 wood stud above the water line ($9.13E^{-05}$) followed by the front section below the water line ($2.77E^{-05}$) and the rear of the wood stud below the water line ($1.50E^{-05}$). The other components contained little or no *A. terreus*.

www.manaraa.com

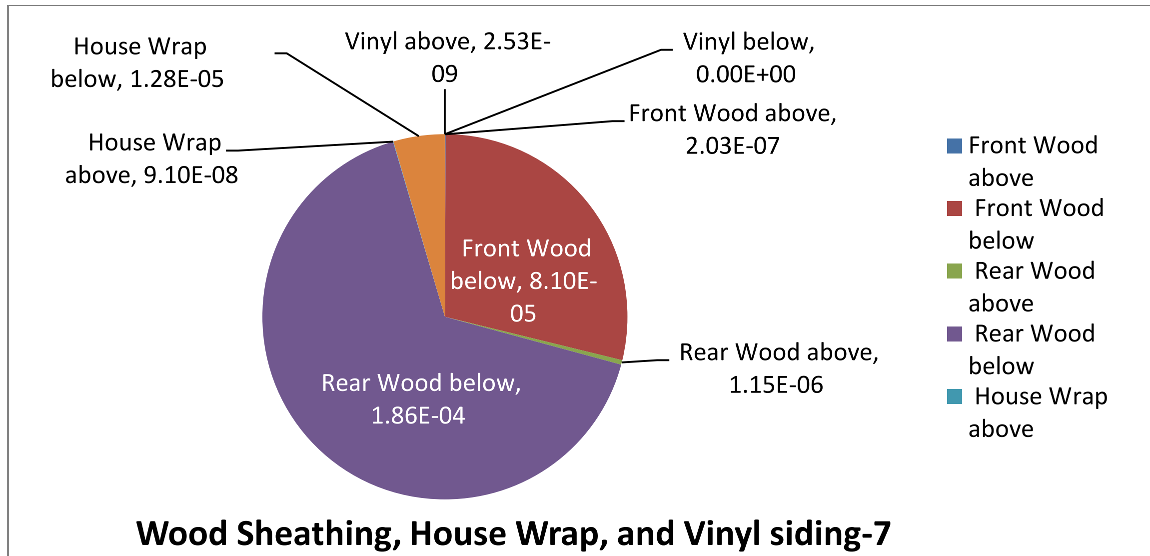

Figure 3.30 The distribution of *Aspergillus terreus*, based on DNA concentrations, on the different components of wet wood sheathing, plus the house wrap, and vinyl siding-7.

The distribution of *Aspergillus terreus* on wood sheathing, house wrap, and vinyl siding in Figure 3.30 shows that this species was present in highest concentration on the wood sheathing. The rear side of the wood sheathing below the water line supported the highest concentration of $1.86E^{-04}$, while the wood sheathing on the front side below the water line supported a concentration of $8.10E^{-05}$. The house wrap below the water line contained the only other notable concentration of *A. terreus* at $1.28E^{-05}$. All other components including the vinyl siding contained very low to no *A. terreus* DNA. Statistics were performed on the wet materials for both above and below the water line in Table 3.13.

المنارة للاستشارات
www.manaraa.com

Table 3.13 Statistical analysis on *Aspergillus terreus* both above and below the water line on wet materials.

State	Waterline	Samples	Mean	Std Dev
wet	above	57	0.000148	0.000518
wet	below	57	2.2E-05	4.65E-05

Table 3.13 shows that *Aspergillus terreus* was present in higher concentrations above the water line on the wet material than below the water line. The dry samples were examined for the presence of *Aspergillus terreus* on the wood stud and gypsum wallboard and are shown in Figure 3.31.

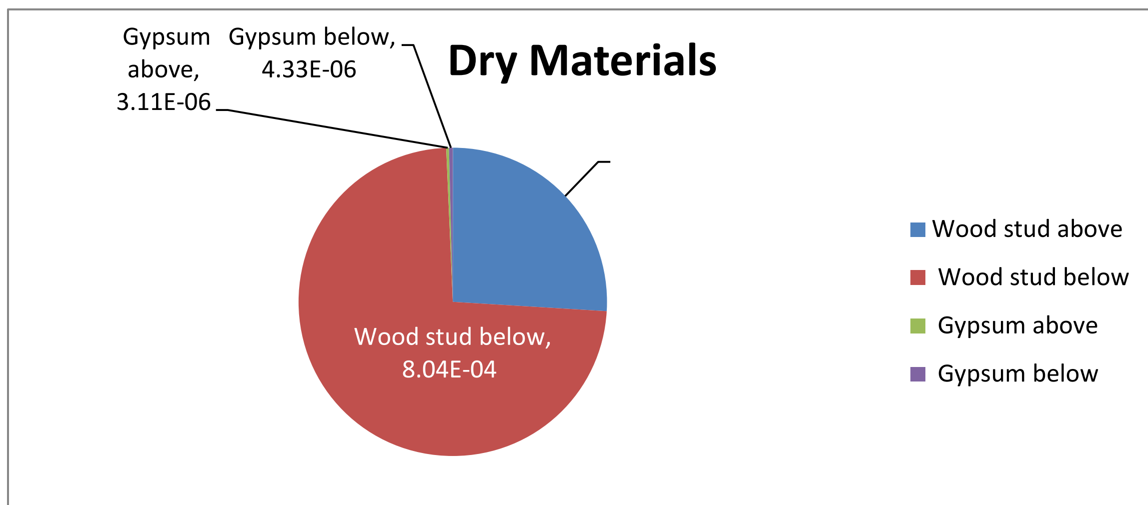

Figure 3.31 The distribution of *Aspergillus terreus*, based on DNA concentrations, on the different components of the dry building materials.

Figure 3.31 shows that *Aspergillus terreus* was present on both of the dry materials although most *A. terreus* was located on the wood stud. The wood stud below the water line had the highest concentration of $8.04E^{-04}$ followed by the wood stud above the water line ($2.85E^{-04}$). The gypsum wallboard above the water line ($3.11E^{-06}$) and

المنارة للاستشارات
www.manaraa.com

gypsum below (4.33E^{-06}) supported the lowest concentrations of *Aspergillus terreus* on the dry materials. There were no statistical differences found in *A. terreus* concentrations among the different dry materials (Table 3.14).

Table 3.14 Statistical (ANOVA) anaylsis of the significant difference of dry building materials. (Means with the same letter are not significantly different).

ANOVA Statisitical Difference			
Tukey Grouping	**Mean**	**N**	**Material**
A	0.0005446	18	Wood stud
A	0.0000037	18	Gypsum

The presence of *Aspergillus terreus* was examined on individual components of the dry samples and these data are shown in Figures 3.33 and Figure 3.34.

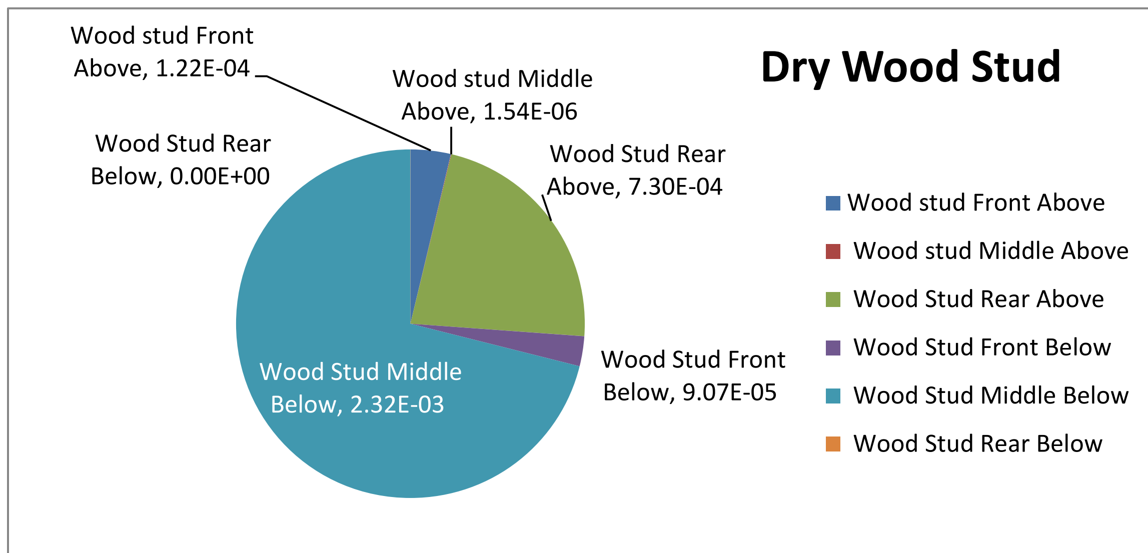

Figure 3.32 The distribution of *Aspergillus terreus*, based on DNA concentrations, on the different section of the dry wood stud.

المنارة للاستشارات
www.manaraa.com

The dry wood stud (Figure 3.32) supported *Aspergillus terreus* in concentrations of 2.32E^{-03} on the middle section of the wood stud below the water line while 7.30E^{-04} was detected on the rear section of the wood stud above the water line. Moderate to low concentrations were also found on the front section of the 2x4 wood stud below the water line (9.07E^{-05}), the front above the water line (1.22E^{-04}), and the middle section above the water line (1.54E^{-06}).

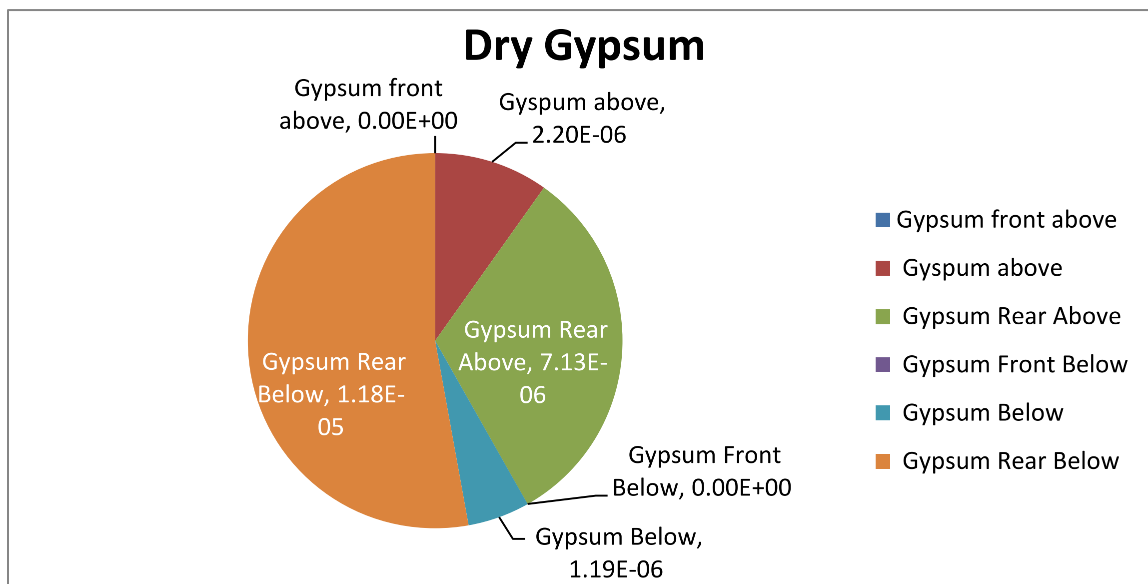

Figure 3.33 The distribution of *Aspergillus terreus*, based on DNA concentrations, on the different components of the dry gypsum.

In Figure 3.33 *Aspergillus terreus* was found in moderate concentrations on the paper rear of the gypsum wallboard below the water line (1.18E^{-05}), followed by gypsum above the water line (2.20E^{-06}), the paper rear above the water line (7.13E^{-06}), and the gypsum below the water line (1.19E^{-06}). Interestingly, there was no indication of *Aspergillus terreus* on the paper front both above and below the water line.

المنارة للاستشارات
www.manaraa.com

Although considered uncommon on building materials, *Aspergillus terreus* was detected on most all of the wet and dry wall materials analyzed in this study in moderate to moderately low concentrations. The batt insulation supported the highest concentration of the wet materials, both as an average as well as an individual concentration of *A. terreus*, were it was found only above the water line. In contrast, *A. terreus* was found in a moderate average concentration of $6.72E^{-05}$ on the wood sheathing but only below the water line. The batt and the wood sheathing came from the small wall unit and would have been close contact with each other. *A. terreus* was also detected in moderate to low concentrations on the wet gypsum boards. Statistical analysis of the wet materials showed that *A. terreus* was 5x more likely to be found above the water line versus below the water line. In addition to the wet samples *Aspergillus terreus* was found in the highest single concentration on the dry wood stud below the water line and was present in moderate levels both above and below the water line on the gypsum and other wood stud locations. Like *A. fumigatus*, it is important to note the ability *A. terreus* to remain in high concentrations on the dry materials seven months after the flood.

Aspergillus versicolor

Aspergillus versicolor is a halo-tolerant mold and can also tolerate low pH. *Aspergillus versicolor* grows at lower temperatures than most other *Aspergillus* species. It occurs worldwide in soil and is very common in indoor environments (Samson et al. 2010). *Aspergillus versicolor* is known to be the major producer of the hepatotoxic and carcinogenic mycotoxin sterigmatocystin (Engelhart et al. 2002), which it can produce on building materials including gypsum board (Flannagan et al. 2011). *Aspergillus versicolor* is in the phylum Ascomycota.The colonies of *A. versicolor* can vary in color at

المنارة للاستشارات
www.manaraa.com

first white, then changing to yellow, orange-yellow to yellow green, often intermixed with flesh to pink colors. The DNA concentrations of *Aspergillus versicolor* was determined using real-time PCR of each wall construction material and converted to the amount of fungal DNA in nannograms per weight of the material sample (Table 3.15).

Table 3.15 Real-time PCR results of *Aspergillus versicolor* DNA in nannograms per weight of material

Sample Code	Material Weight (mg)	Extraction volume	Amount µL used	Real Time (ng)	Average Reps	(ng)DNA/(mg) Material	Average DNA/Material
B1AP1	100	80	3 µL	2.52E-04		6.72E-05	
B1AP2	100	80	3 µL	0.00E+00	8.96E-05	0.00E+00	2.39E-05
B1AP3	100	80	3 µL	1.68E-05		4.48E-06	
B1BP1	140	80	3 µL	0.00E+00		0.00E+00	
B1BP2	140	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
B1BP3	140	80	3 µL	0.00E+00		0.00E+00	
B1AN1	50	80	3 µL	5.75E-05		3.07E-05	
B1AN2	50	80	3 µL	5.65E-07	1.99E-05	3.01E-07	1.06E-05
B1AN3	50	80	3 µL	1.65E-06		8.80E-07	
B1BN1	100	80	3 µL	1.23E-04		3.28E-05	
B1BN2	100	80	3 µL	1.02E-04	1.39E-04	2.72E-05	3.71E-05
B1BN3	100	80	3 µL	1.92E-04		5.12E-05	
B1AF1	50	80	3 µL	1.23E-06		6.56E-07	
B1AF2	50	80	3 µL	0.00E+00	4.10E-07	0.00E+00	2.19E-07
B1BF1	100	80	3 µL	0.00E+00		0.00E+00	
B1BF2	100	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
B1BF3	100	80	3 µL	0.00E+00		0.00E+00	
G3AS1	200	80	3 µL	6.01E-03		8.02E-04	
G3AS2	200	80	3 µL	1.13E-06	2.04E-03	1.51E-07	2.72E-04
G3AS3	200	80	3 µL	9.65E-05		1.29E-05	
G3BS1	250	80	3 µL	1.41E-07		1.50E-08	
G3BS2	250	80	3 µL	1.66E-04	1.05E-04	1.77E-05	1.12E-05
G3BS3	250	80	3 µL	1.50E-04		1.60E-05	
G3AR1	250	80	3 µL	0.00E+00		0.00E+00	
G3AR2	250	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00

المنارة للاستشارات
www.manaraa.com

Table 3.15 (continued)

G3AR3	250	80	3 µL	0.00E+00		0.00E+00	
G3BR1	300	80	3 µL	1.79E-05		1.59E-06	
G3BR2	300	80	3 µL	1.07E-05	1.42E-05	9.51E-07	1.27E-06
G3BR3	300	80	3 µL	1.41E-05		1.25E-06	
G3AM1	500	80	3 µL	1.41E-06		7.52E-08	
G3AM2	500	80	3 µL	7.70E-07	1.27E-06	4.11E-08	6.79E-08
G3AM3	500	80	3 µL	1.64E-06		8.75E-08	
G3BM1	500	80	3 µL	2.41E-07		1.29E-08	
G3BM2	500	80	3 µL	0.00E+00	8.03E-08	0.00E+00	4.28E-09
G3BM3	500	80	3 µL	0.00E+00		0.00E+00	
G4AS1	200	80	3 µL	8.61E-06		1.15E-06	
G4AS2	230	80	3 µL	0.00E+00	1.84E-05	0.00E+00	2.46E-06
G4AS3	200	80	3 µL	4.67E-05		6.23E-06	
G4BS1	210	80	3 µL	5.94E-05		7.54E-06	
G4BS2	200	80	3 µL	2.66E-04	1.33E-04	3.55E-05	1.76E-05
G4BS3	200	80	3 µL	7.41E-05		9.88E-06	
G4AR1	100	80	3 µL	2.63E-07		7.01E-08	
G4AR2	100	80	3 µL	1.78E-05	1.00E-05	4.75E-06	2.67E-06
G4AR3	100	80	3 µL	1.20E-05		3.20E-06	
G4BR1	200	80	3 µL	1.28E-05		1.71E-06	
G4BR2	200	80	3 µL	9.32E-07	4.76E-06	1.24E-07	6.34E-07
G4BR3	200	80	3 µL	5.37E-07		7.16E-08	
G4AM1	300	80	3 µL	9.61E-07		8.54E-08	
G4AM2	300	80	3 µL	0.00E+00	4.56E-07	0.00E+00	3.98E-08
G4AM3	320	80	3 µL	4.07E-07		3.39E-08	
G4BM1	500	80	3 µL	4.03E-07		2.15E-08	
G4BM2	520	80	3 µL	8.22E-07	5.42E-07	4.22E-08	2.84E-08
G4BM3	500	80	3 µL	4.02E-07		2.14E-08	
G2TS1	150	80	3 µL	1.12E-05		1.99E-06	
G2TS2	160	80	3 µL	0.00E+00	4.89E-06	0.00E+00	8.69E-07
G2TS3	150	80	3 µL	3.46E-06		6.15E-07	
G2BS1	160	80	3 µL	0.00E+00		0.00E+00	
G2BS2	150	80	3 µL	0.00E+00	5.73E-06	0.00E+00	1.02E-06
G2BS3	150	80	3 µL	1.72E-05		3.06E-06	
G2TR1	100	80	3 µL	2.90E-04		7.73E-05	
G2TR2	100	80	3 µL	0.00E+00	1.15E-04	0.00E+00	3.07E-05
G2TR3	100	80	3 µL	5.49E-05		1.46E-05	
G2BR1	100	80	3 µL	3.58E-05		9.55E-06	

المنارة للاستشارات
www.manaraa.com

Table 3.15 (continued)

G2BR2	80	80	3 μL	2.28E-05	2.23E-05	7.60E-06	6.62E-06
G2BR3	80	80	3 μL	8.15E-06		2.72E-06	
G2TM1	3150	80	3 μL	4.08E-07		3.45E-09	
G2TM2	100	80	3 μL	0.00E+00	2.22E-07	0.00E+00	1.27E-08
G2TM3	200	80	3 μL	2.59E-07		3.45E-08	
G2BM1	170	80	3 μL	2.79E-07		4.38E-08	
G2BM2	280	80	3 μL	7.79E-07	5.29E-07	7.42E-08	6.28E-08
G2BM3	200	80	3 μL	5.28E-07		7.04E-08	
W7AS1	100	80	3 μL	1.56E-06		4.16E-07	
W7AS2	100	80	3 μL	1.45E-06	1.26E-06	3.87E-07	3.16E-07
W7AS3	140	80	3 μL	7.59E-07		1.45E-07	
W7BS1	50	80	3 μL	3.10E-06		1.65E-06	
W7BS2	50	80	3 μL	2.34E-04	2.36E-04	1.25E-04	1.47E-04
W7BS3	40	80	3 μL	4.71E-04		3.14E-04	
W7AR1	40	80	3 μL	2.29E-04		1.53E-04	
W7AR2	80	80	3 μL	1.57E-06	7.71E-05	5.23E-07	5.11E-05
W7AR3	80	80	3 μL	6.52E-07		2.17E-07	
W7BR1	50	80	3 μL	2.71E-07		1.45E-07	
W7BR2	60	80	3 μL	0.00E+00	9.03E-08	0.00E+00	4.82E-08
W7BR3	50	80	3 μL	0.00E+00		0.00E+00	
I7A1	40	80	3 μL	0.00E+00		0.00E+00	
I7A2	40	80	3 μL	5.01E-07	2.57E-07	3.34E-07	1.71E-07
I7A3	40	80	3 μL	2.69E-07		1.79E-07	
I7B1	60	80	3 μL	3.84E-07		1.71E-07	
I7B2	50	80	3 μL	0.00E+00	1.28E-07	0.00E+00	5.69E-08
I7B3	50	80	3 μL	0.00E+00		0.00E+00	
V7A1	400	80	3 μL	0.00E+00		0.00E+00	
V7A2	400	80	3 μL	4.27E-07	4.23E-07	2.85E-08	2.82E-08
V7A3	400	80	3 μL	8.43E-07		5.62E-08	
V7B1	400	80	3 μL	2.20E-07		1.47E-08	
V7B2	400	80	3 μL	2.24E-07	4.46E-07	1.49E-08	3.64E-08
V7B3	300	80	3 μL	8.95E-07		7.96E-08	
W4AS1	80	80	3 μL	1.24E-06		4.13E-07	
W4AS2	90	80	3 μL	7.08E-05	5.83E-05	2.10E-05	8.34E-06
W4AS3	760	80	3 μL	1.03E-04		3.61E-06	
W4BS1	900	80	3 μL	1.13E-04		3.35E-06	
W4BS2	76	80	3 μL	1.52E-05	6.72E-05	5.33E-06	7.91E-06
W4BS3	130	80	3 μL	7.34E-05		1.51E-05	

المنارة للاستشارات
www.manaraa.com

Talbe 3.15 (continued)

W4BR1	150	80	3 µL	1.21E-05		2.15E-06	
W4AR2	90	80	3 µL	6.93E-06	8.17E-06	2.05E-06	2.10E-06
W4AR3	70	80	3 µL	5.47E-06		2.08E-06	
W4BR1	40	80	3 µL	2.58E-06		1.72E-06	
W4BR2	65	80	3 µL	3.66E-05	5.31E-05	1.50E-05	2.69E-05
W4BR3	50	80	3 µL	1.20E-04		6.40E-05	
W4AM1	60	80	3 µL	1.26E-04		5.60E-05	
W4AM2	50	80	3 µL	1.26E-06	4.28E-05	6.72E-07	1.92E-05
W4AM3	40	80	3 µL	1.19E-06		7.93E-07	
W4BM1	53	80	3 µL	7.26E-07		3.65E-07	
W4BM2	50	80	3 µL	3.32E-07	6.03E-07	1.77E-07	2.82E-07
W4BM3	66	80	3 µL	7.51E-07		3.03E-07	
G1AS1	120	80	3 µL	3.86E-07		8.58E-08	
G1AS2	170	80	3 µL	0.00E+00	1.29E-07	0.00E+00	2.86E-08
G1AS3	200	80	3 µL	0.00E+00		0.00E+00	
G1BS1	200	80	3 µL	0.00E+00		0.00E+00	
G1BS2	300	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G1BS3	500	80	3 µL	0.00E+00		0.00E+00	
G1AR1	62	80	3 µL	1.66E-07		7.14E-08	
G1AR2	75	80	3 µL	9.75E-08	1.08E-07	3.47E-08	4.08E-08
G1AR3	100	80	3 µL	6.08E-08		1.62E-08	
G1BR1	97	80	3 µL	2.54E-07		6.98E-08	
G1BR2	100	80	3 µL	2.17E-07	4.13E-07	5.79E-08	1.73E-07
G1BR3	150	80	3 µL	7.69E-07		1.37E-07	
G1AM1	500	80	3 µL	7.04E-08		3.75E-09	
G1AM2	430	80	3 µL	7.69E-08	6.11E-08	4.77E-09	3.59E-09
G1AM3	427	80	3 µL	3.61E-08		2.25E-09	
G1BM1	450	80	3 µL	N/A		N/A	
G1BM2	360	80	3 µL	1.63E-07	1.61E-07	1.21E-08	9.96E-09
G1BM3	540	80	3 µL	1.59E-07		7.85E-09	
S1AF1	50	80	3 µL	1.23E-04		6.56E-05	
S1AF2	50	80	3 µL	2.35E-05	6.12E-05	1.25E-05	3.26E-05
S1AF3	50	80	3 µL	3.70E-05		1.97E-05	
S1AM1	50	80	3 µL	2.80E-05		1.49E-05	
S1AM2	50	80	3 µL	1.66E-04	6.53E-05	8.85E-05	3.48E-05
S1AM3	60	80	3 µL	1.99E-06		8.84E-07	
S1AR1	50	80	3 µL	2.95E-04		1.57E-04	
S1AR2	50	80	3 µL	2.25E-05	1.07E-04	1.20E-05	5.69E-05

المنارة للاستشارات
www.manaraa.com

Table 3.15 (continued)

S1AR3	50	80	3 µL	2.29E-06		1.22E-06	
S2BF1	50	80	3 µL	1.13E-03		6.03E-04	
S2BF2	60	80	3 µL	9.47E-04	1.15E-03	4.21E-04	5.87E-04
S2BF3	50	80	3 µL	1.38E-03		7.36E-04	
S2BM1	60	80	3 µL	9.19E-07		4.08E-07	
S2BM2	50	80	3 µL	5.08E-06	2.17E-06	2.71E-06	1.11E-06
S2BM3	60	80	3 µL	5.10E-07		2.27E-07	
S2BR1	50	80	3 µL	0.00E+00		0.00E+00	
S2BR2	50	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
S2BR3	50	80	3 µL	0.00E+00		0.00E+00	
G3AF1	200	80	3 µL	0.00E+00		0.00E+00	
G3AF2	100	80	3 µL	1.78E-07	1.16E-07	4.75E-08	2.34E-08
G3AF3	200	80	3 µL	1.70E-07		2.27E-08	
G3AM1	200	80	3 µL	5.23E-07		6.97E-08	
G3AM2	200	80	3 µL	1.01E-07	5.39E-07	1.35E-08	7.19E-08
G3AM3	200	80	3 µL	9.94E-07		1.33E-07	
G3AR1	100	80	3 µL	2.88E-07		7.68E-08	
G3AR2	100	80	3 µL	1.62E-05	1.11E-05	4.32E-06	2.97E-06
G3AR3	100	80	3 µL	1.69E-05		4.51E-06	
G4BF1	100	80	3 µL	0.00E+00		0.00E+00	
G4BF2	200	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G4BF3	200	80	3 µL	0.00E+00		0.00E+00	
G4BM1	300	80	3 µL	3.65E-08		3.24E-09	
G4BM2	200	80	3 µL	3.22E-06	1.12E-06	4.29E-07	1.49E-07
G4BM3	200	80	3 µL	1.13E-07		1.51E-08	
G4BR1	100	80	3 µL	7.38E-04		1.97E-04	
G4BR2	100	80	3 µL	1.30E-04	4.47E-04	3.47E-05	1.19E-04
G4BR3	100	80	3 µL	4.72E-04		1.26E-04	

The wet building materials were analyzed for *Aspergillus versicolor* on each type of material and this data are shown in Figure 3.34.

المنارة للاستشارات
www.manaraa.com

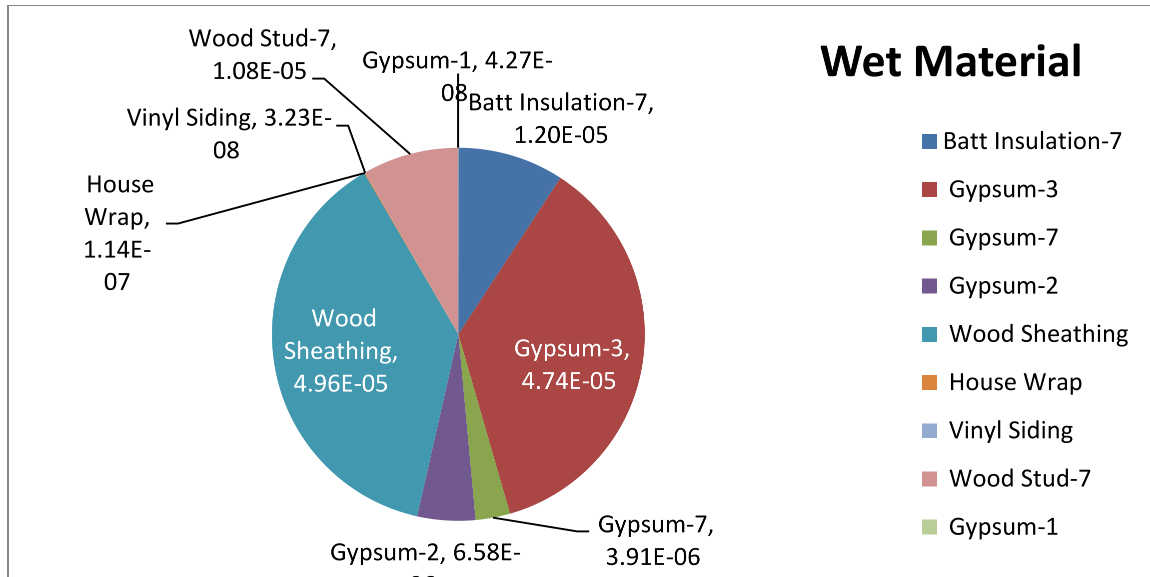

Figure 3.34 The distribution of *Aspergillus versicolor*, based on averaged DNA concentrations, on the different wet building materials. Samples with a 7 came from the same wall unit.

The wet material averages for *Aspergillus versicolor* (Figure 3.34) shows that the mold was detected in all materials. The wood sheathing had the highest concentration of $4.96E^{-05}$ closely followed by gypsum-3 ($4.74E^{-05}$), batt insulation ($1.20E^{-05}$), and wood stud-7 ($1.08E^{-05}$). Two other gypsum boards contained moderately low levels of *A. versicolor*, gypsum-2 ($6.58E^{-06}$) and gypsum-7 ($3.91E^{-06}$), while the other materials supported low to very low concentrations. There were no statistical differences found in *A. versicolor* concentrations among the different wet materials (Table 3.16).

المنارة للاستشارات
www.manaraa.com

Table 3.16 Statistical (ANOVA) anaylsis of the significant difference of wet building materials. (Means with the same letter are not significantly different).

ANOVA Statistical Difference			
Tukey Grouping	**Mean**	**N**	**Material**
A	0.00004958	12	Wood sheathing
A	0.00004735	18	Gypsum-3
A	0.00001197	18	Batt Insulation
A	0.00001078	18	Wood stud
A	0.00000654	18	Gypsum-2
A	0.00000391	18	Gypsum-7
A	0.00000011	6	House wrap
A	0.00000003	6	Vinyl siding
A	0.00000003	17	Gypsum-1

The presence of *Aspergillus versicolor* was examined on individual components of each material and these data are shown in Figures 3.35-3.41.

المنارة للاستشارات
www.manaraa.com

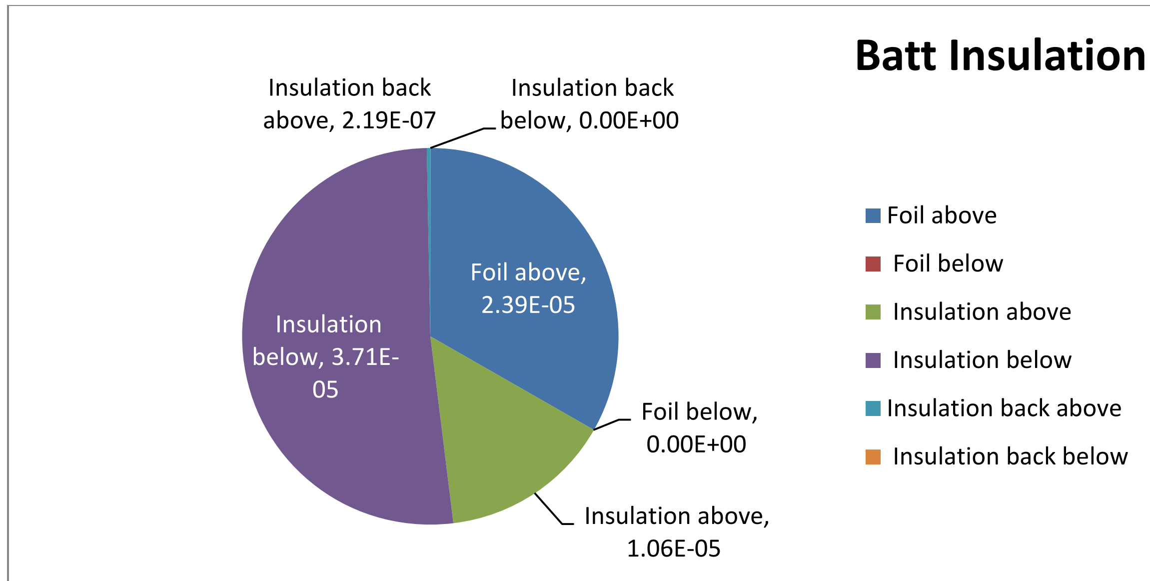

Figure 3.35 The distribution of *Aspergillus versicolor*, based on DNA concentrations, on the different components of the wet batt insulation-7.

The distribution of *Aspergillus versicolor* in the batt insulation (Figure 3.35) ranged from highest concentration of $3.71E^{-05}$ below the water line on the insulation (purple) followed by the foil above the water line ($2.39E^{-05}$), and the insulation above the water line ($1.06E^{-05}$). It is interesting to note that *Aspergillus versicolor* occurred on the foil above the water line and wasn't identified on the foil below the water line. There was little to no *A. versicolor* on the back side of the insulation both above and below the water line.

المنارة للاستشارات
www.manaraa.com

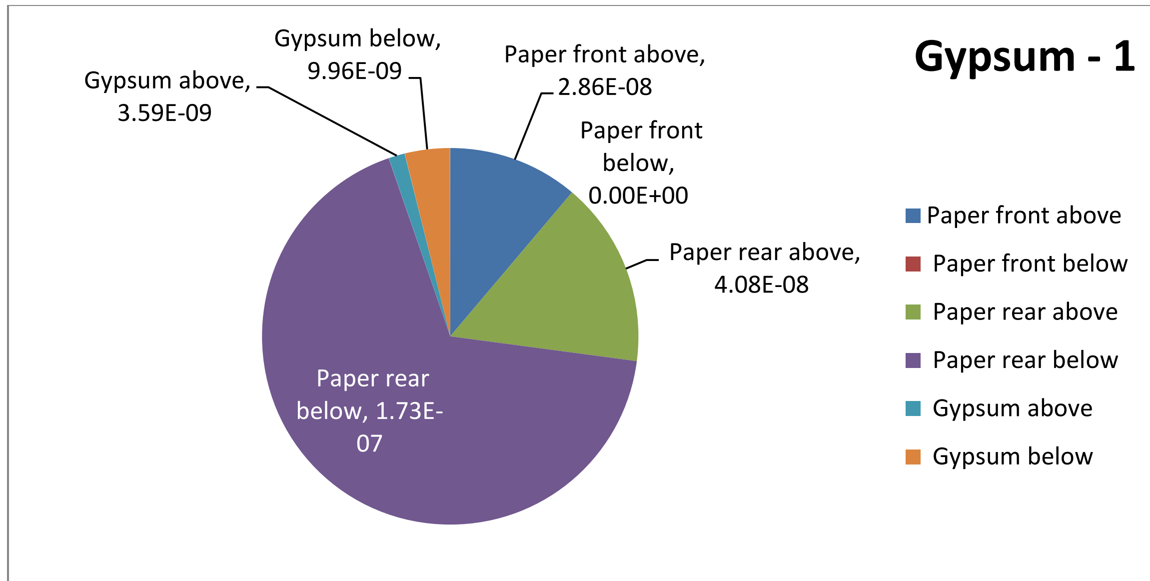

Figure 3.36 The distribution of *Aspergillus versicolor*, based on DNA concentrations, on the different components of the gypsum-1.

Figure 3.36 shows a presence of *Aspergillus versicolor* in low concentrations to no concentrations in all locations of the gypsum-1 wallboard. However, the majority of *Aspergillus versicolor* was found on the rear paper above the water ($1.73E^{-07}$) and below the water line $4.08E^{-08}$.

المنارة للاستشارات
www.manaraa.com

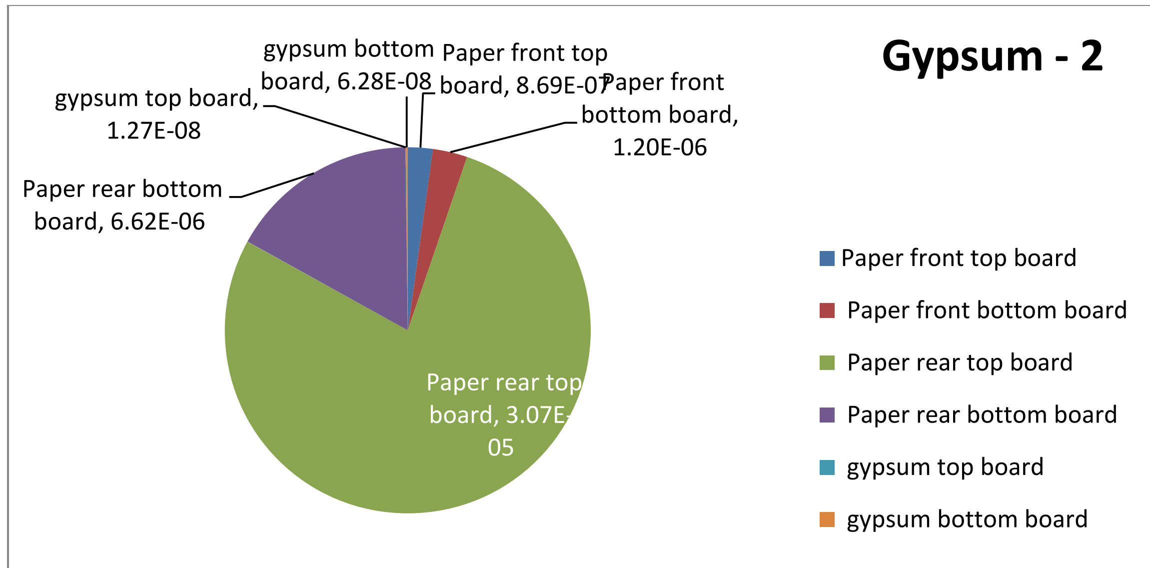

Figure 3.37 The distribution of *Aspergillus versicolor*, based on DNA concentrations, on the different components of wet gypsum-2.

Aspergillus versicolor was found on gypsum-2 (Figure 3.37) in moderate concentrations on the rear paper on the top board ($3.07E^{-05}$) followed by the rear the paper bottom of board ($6.62E^{-06}$). The paper front bottom ($1.20E^{-06}$) also contained moderately low concentrations of *Aspergillus versicolor*. Finally, *Aspergillus versicolor* was detected at very low concentrations on the paper front top of the board ($8.69E^{-07}$) and gypsum material at the bottom ($6.28E^{-08}$) and top of the board ($1.27E^{-08}$).

المنارة للاستشارات
www.manaraa.com

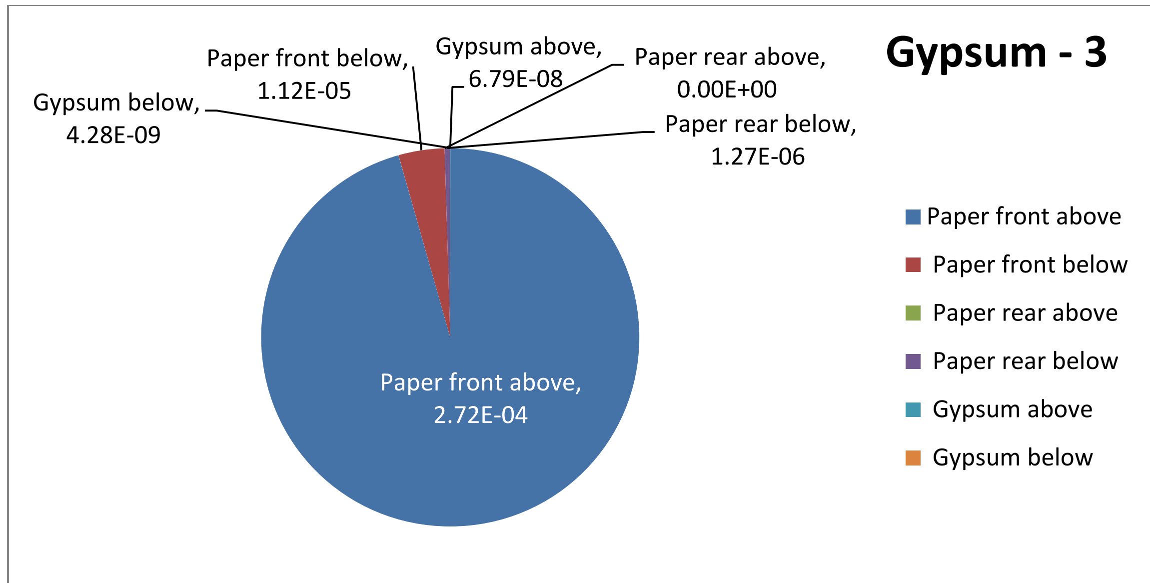

Figure 3.38 The distribution of *Aspergillus versicolor*, based on DNA concentrations, on the different components of the wet gypsum-3.

Gypsum -3 (Figure 3.38) supported *Aspergillus versicolor* in moderately high concentrations on the paper front above the water line ($2.72E^{-04}$) while the paper front below the water line ($1.12E^{-05}$) was the next highest concentration. Also the other gypsum components had low to no concentrations including the paper rear below the water line ($1.27E^{-06}$).

المنارة للاستشارات
www.manaraa.com

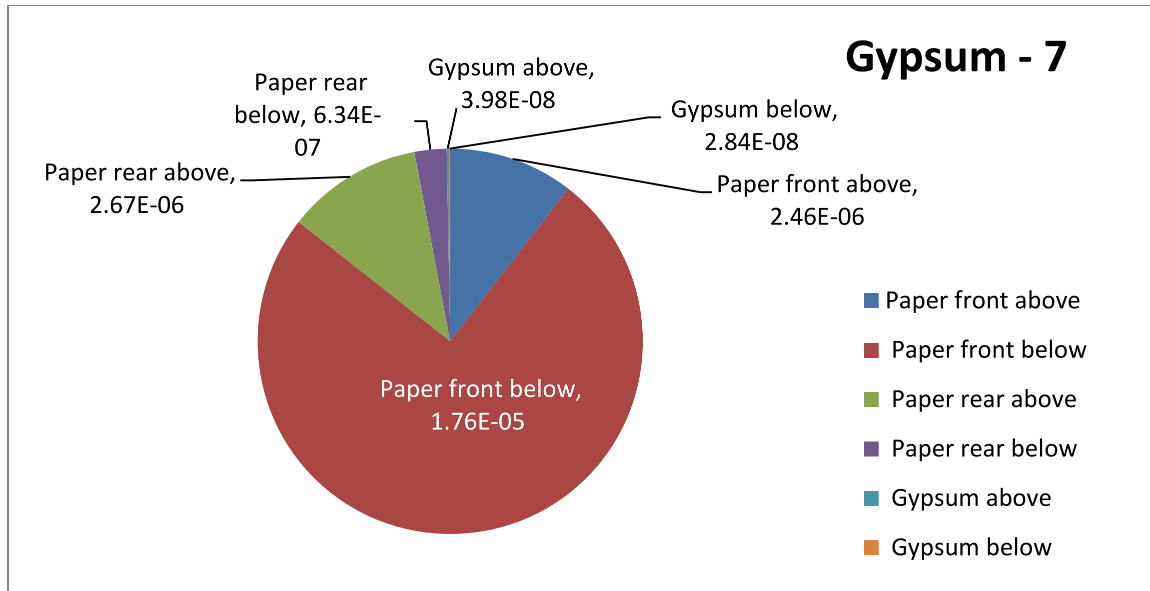

Figure 3.39 The distribution of *Aspergillus versicolor*, based on DNA concentrations, on the different components of the wet gypsum-7.

Gypsum -7 (Figure 3.39) contained *Aspergillus versicolor* in moderately low concentrations on the paper front below the water line (1.76E^{-05}), paper front above the water line (2.46E^{-06}), and the paper rear above the water line (2.67E^{-06}). All other components of gypsum-7 board supported low to no *A. versicolor*.

المنارة للاستشارات
www.manaraa.com

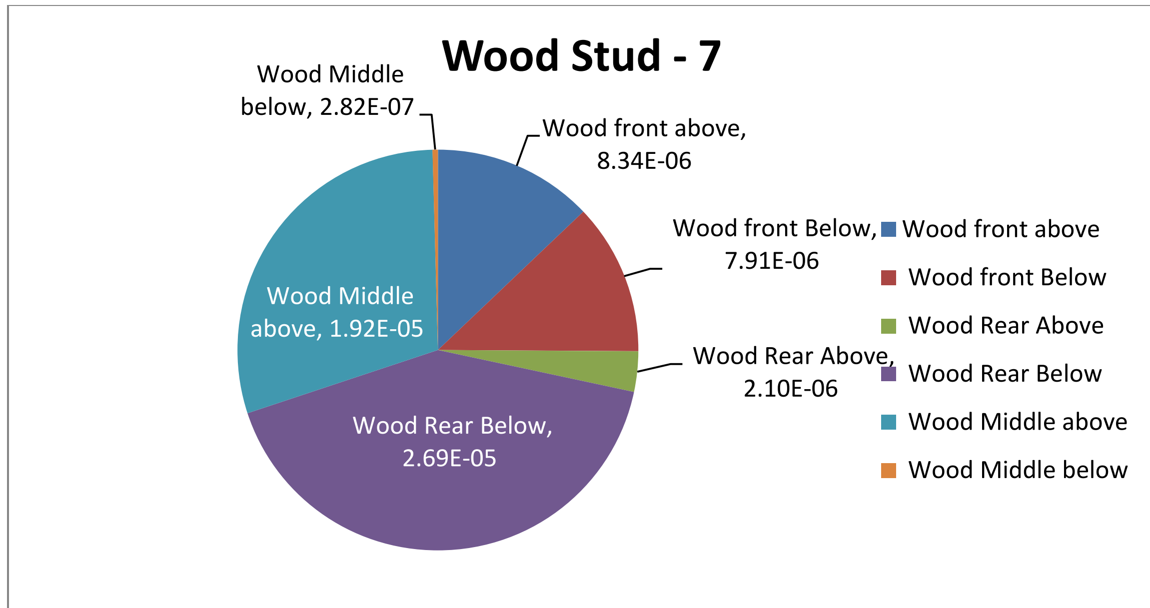

Figure 3.40 The distribution of *Aspergillus versicolor*, based on DNA concentrations, on the different components of the wet wood stud-7.

Figure 3.40 shows that *Aspergillus versicolor* was detected in moderately low to low levels in all samples of the 2x4 wood stud. The highest concentration was found on the rear section of the stud below the water line ($2.69E^{-05}$) followed by the middle section above the water line ($1.92E^{-05}$), the front of the wood stud below ($7.91E^{-06}$) and above ($8.34E^{-06}$) the water line, and the rear above the water line ($2.10E^{-06}$).

www.manaraa.com

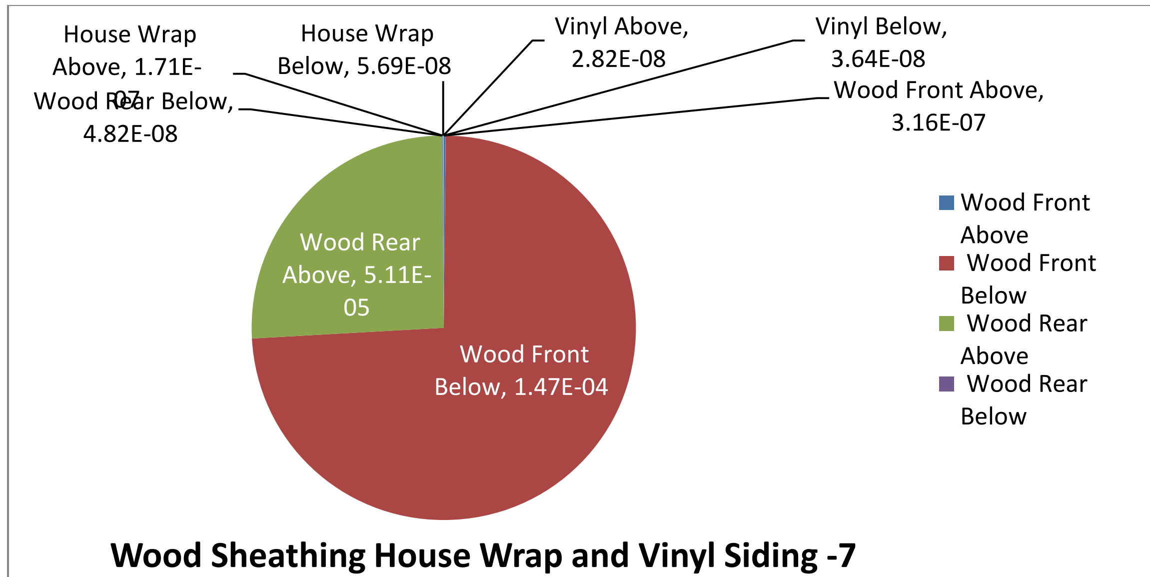

Figure 3.41 The distribution of *Aspergillus versicolor*, based on DNA concentrations, on the different components for the wet wood sheathing, plus house wrap, and vinyl siding-7 building.

Aspergillus versicolor was detected in all samples of the wood sheathing, house wrap, and vinyl siding-7 (Figure 3.41). However, concentrations of this mold were much greater in the wood sheathing than in the other two wall materials. The front of the wood sheathing below the water line contained the highest concentration ($1.47E^{-04}$) followed by the rear of the wood sheathing above the water line ($5.11E^{-05}$). All other material and other wood sheathing components supported very low to no levels of *A. versicolor*. Statistics was performed for the wet materials on both above and below the water line for *A. versicolor* and these results are given in Table 3.17.

المنارة للاستشارات
www.manaraa.com

Table 3.17 Statistical analysis on *Aspergillus versicolor* both above and below the water line on all wet materials.

State	Waterline	Samples	Mean	Std dev
wet	above	57	2.07E-05	0.000108
wet	below	56	1.34E-05	4.58E-05

Table 3.17 shows that *Aspergillus versicolor* was present in higher concentrations above the water line than below the water line for all wet materials. However, the difference between above and below was quite small. The dry samples were also examined for *Aspergillus versicolor* on the wood stud and gypsum wallboard. The different dry materials examined for the mold species both above and below the water line are shown in Figure 3.42.

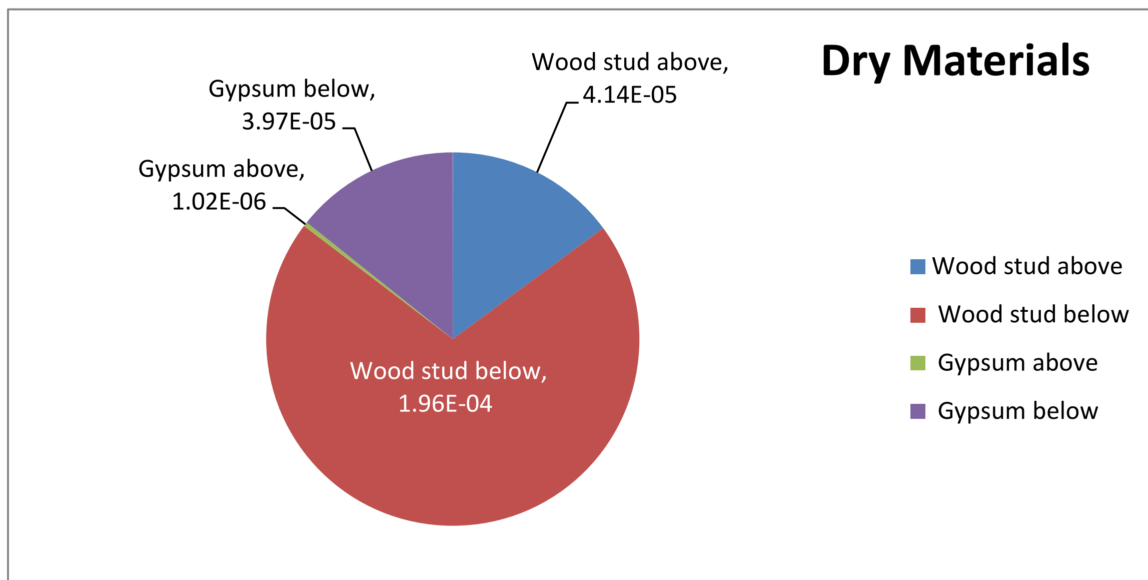

Figure 3.42 The distribution of *Aspergillus versicolor*, based on DNA concentrations, on the different dry building materials.

المنارة للاستشارات
www.manaraa.com

Figure 3.42 shows that *Aspergillus versicolor* was present on all dry materials. The wood stud below the water line supported the highest concentration at $1.96E^{-04}$ followed by gypsum below the water line ($3.97E^{-05}$). The gypsum above the water line ($1.02E^{-06}$) and the wood stud above the water line had lower concentrations ($4.14E^{-05}$). There were no statistical differences found in *A. versicolor* concentrations among the different dry materials (Table 3.18).

Table 3.18 Statistical (ANOVA) anaylsis of the significant difference of dry building materials. (Means with the same letter are not significantly different).

ANOVA Statistical Differences			
Tukey Grouping	**Mean**	**N**	**Material**
A	0.00011865	18	Wood stud
A	0.00002039	18	Gypsum

The concentrations of *Aspergillus versicolor* were examined on individual components of these materials and are shown in Figures 3.43 and Figure 3.44.

المنارة للاستشارات
www.manaraa.com

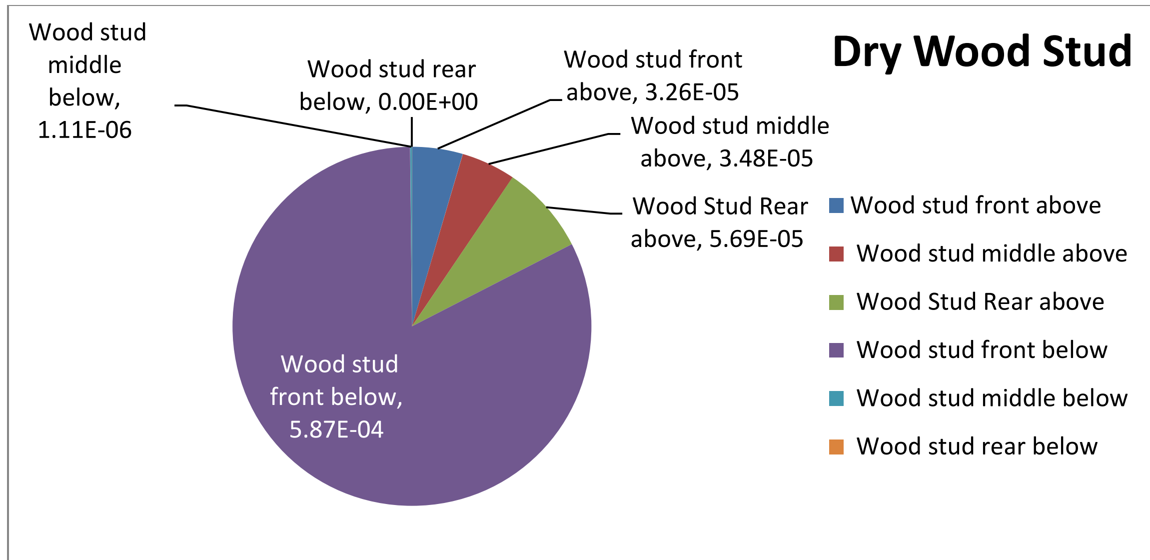

Figure 3.43 The distribution of *Aspergillus versicolor*, based on DNA concentrations, on the different components of the dry wood stud.

Figure 3.43 shows that *Aspergillus versicolor* was primarily present on the front section of the 2x4 wood stud below the water line in moderate concentrations ($5.87E^{-04}$). Lower concentrations were also detected on the rear section of the wood stud above the water line ($5.69E^{-05}$), the front section of the wood stud above the water line ($3.26E^{-05}$), the wood stud middle above the water line ($3.48E^{-05}$), and the middle section of the wood stud below the water line ($1.11E^{-06}$).

المنارة للاستشارات
www.manaraa.com

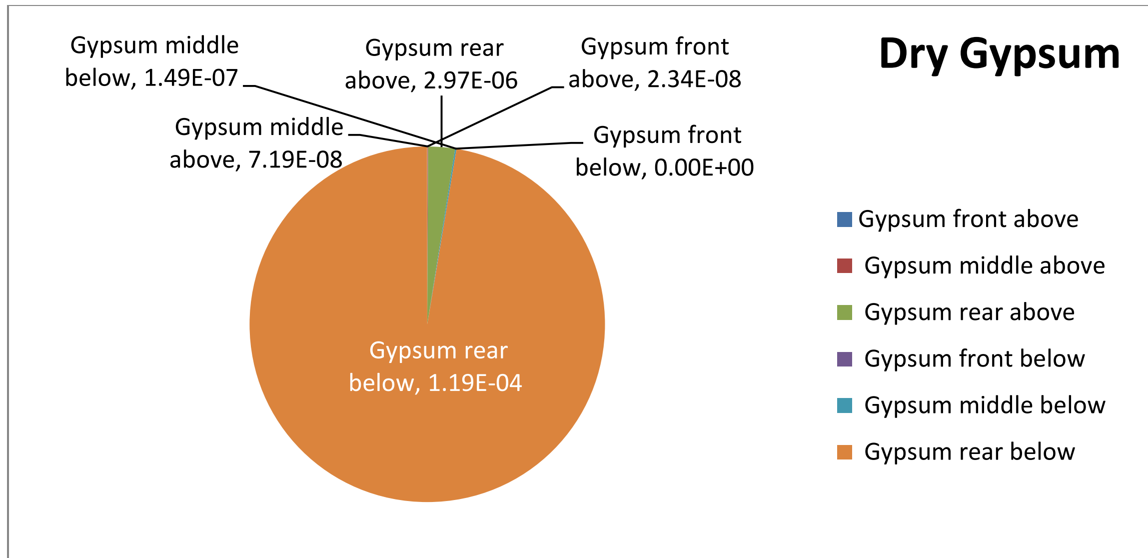

Figure 3.44 The distribution of *Aspergillus versicolor*, based on DNA concentrations, on the different components of the dry gypsum.

The majority of the *Aspergillus versicolor* was found on the dry gypsum and located on the rear paper below the water line (Figure 3.44) at a concentration of $1.19E^{-04}$. The rear paper above the water line also supported moderately low levels of this fungus. All other components of the gypsum contained very low to no concentrations of *A. versicolor*.

Aspergillus versicolor was detected both above and below the water line on the different building materials with little preference exhibited to a particular location. Overall, *A. versicolor* was detected in moderate levels on all of the different building materials and did not seem to favor one material over the rest. On the wet materials, the highest averages were found on the wood sheathing, one of the gypsum boards and the wood stud. The highest concentration detected was on the dry wood stud. Moderate concentrations were also detected on the dry gypsum. Like several of the other

المنارة للاستشارات
www.manaraa.com

Aspergillus species, *A. versicolor* appears to survive in notable levels seven months after flooding.

Chaetomium globosum

Chaetomium globosum is a dematiaceous, filamentous fungus belonging to the phylum Ascomycetes. *C. gobosum* can be found in indoor environments on walls and cellulosic materials including wall paper, books, and textiles after water damge. *Chaetomium globosum* is considered to be a cellulotytic species which can grow in optimum temperatures of 18-20°C and can be resistant to UV irradiation. *Chaetomium globosum* perithecia are dark brown to blackish, globose to ovoid, mostly 150-220µm in diameter with dark hyphal appendages. The pertiheicial hairs are numerous, unbranched, and tapered (Samson et al. 2010). The DNA concentrations of *Chaetomium globosum* was determined using real-time PCR of each wall material and converted to the amount of fungal DNA in nannograms per weight of the material sample (Table 3.19).

Table 3.19 Real-time PCR results of *Chaetomium globosum* DNA in nannograms per weight of material

Sample Code	Material Weight (mg)	Extraction volume	Amount µL used	Real Time (ng)	Average Reps	(ng)DNA/(mg) Material	Average DNA/Material
B1AP1	100	80	3 µL	0.00E+00		0.00E+00	
B1AP2	100	80	3 µL	0.00E+00	5.73E-04	0.00E+00	1.53E-04
B1AP3	100	80	3 µL	1.72E-03		4.59E-04	
B1BP1	140	80	3 µL	0.00E+00		0.00E+00	
B1BP2	140	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
B1BP3	140	80	3 µL	0.00E+00		0.00E+00	
B1AN1	50	80	3 µL	7.99E-04		4.26E-04	

المنارة للاستشارات
www.manaraa.com

Table 3.19 (continued)

B1AN2	50	80	3 μL	1.69E-04	3.47E-04	9.01E-05	1.85E-04
B1AN3	50	80	3 μL	7.25E-05		3.87E-05	
B1BN1	100	80	3 μL	0.00E+00		0.00E+00	
B1BN3	100	80	3 μL	0.00E+00		0.00E+00	
B1AF1	50	80	3 μL	1.48E-05		7.89E-06	
B1AF2	50	80	3 μL	0.00E+00	8.07E-06	0.00E+00	4.30E-06
B1AF3	50	80	3 μL	9.41E-06		5.02E-06	
B1BF1	100	80	3 μL	0.00E+00		0.00E+00	
B1BF2	100	80	3 μL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
B1BF3	100	80	3 μL	0.00E+00		0.00E+00	
G3AS1	200	80	3 μL	0.00E+00		0.00E+00	
G3AS2	200	80	3 μL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G3AS3	200	80	3 μL	0.00E+00		0.00E+00	
G3BS1	250	80	3 μL	0.00E+00		0.00E+00	
G3BS2	250	80	3 μL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G3BS3	250	80	3 μL	0.00E+00		0.00E+00	
G3AR1	250	80	3 μL	0.00E+00		0.00E+00	
G3AR2	250	80	3 μL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G3AR3	250	80	3 μL	0.00E+00		0.00E+00	
G3BR1	300	80	3 μL	0.00E+00		0.00E+00	
G3BR2	300	80	3 μL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G3BR3	300	80	3 μL	0.00E+00		0.00E+00	
G3AM1	500	80	3 μL	3.11E-06		1.66E-07	
G3AM2	500	80	3 μL	1.03E-06	1.56E-06	5.49E-08	8.31E-08
G3AM3	500	80	3 μL	5.34E-07		2.85E-08	
G3BM1	500	80	3 μL	1.40E-06		7.47E-08	
G3BM2	500	80	3 μL	9.58E-07	2.36E-06	5.11E-08	4.19E-08
G3BM3	500	80	3 μL	0.00E+00		0.00E+00	
G4AS1	200	80	3 μL	0.00E+00		0.00E+00	
G4AS2	230	80	3 μL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G4AS3	200	80	3 μL	0.00E+00		0.00E+00	
G4BS1	210	80	3 μL	0.00E+00		0.00E+00	
G4BS2	200	80	3 μL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G4BS3	200	80	3 μL	0.00E+00		0.00E+00	
G4AR1	100	80	3 μL	7.71E-05		2.06E-05	
G4AR2	100	80	3 μL	0.00E+00	2.57E-05	0.00E+00	6.85E-06
G4AR3	100	80	3 μL	0.00E+00		0.00E+00	
G4BR1	200	80	3 μL	0.00E+00		0.00E+00	

المنارة للاستشارات
www.manaraa.com

Table 3.19 (continued)

G4BR2	200	80	3 μL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G4BR3	200	80	3 μL	0.00E+00		0.00E+00	
G4AM1	300	80	3 μL	1.02E-05		9.07E-07	
G4AM2	300	80	3 μL	0.00E+00	3.40E-06	0.00E+00	3.02E-07
G4AM3	320	80	3 μL	0.00E+00		0.00E+00	
G4BM1	500	80	3 μL	0.00E+00		0.00E+00	
G4BM2	520	80	3 μL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G4BM3	500	80	3 μL	0.00E+00		0.00E+00	
G2TS1	150	80	3 μL	2.71E-02		4.82E-03	
G2TS2	160	80	3 μL	0.00E+00	2.11E-02	0.00E+00	3.76E-03
G2TS3	150	80	3 μL	3.63E-02		6.45E-03	
G2BS1	160	80	3 μL	5.57E-03		9.28E-04	
G2BS2	150	80	3 μL	4.35E-02	1.64E-02	7.73E-03	2.89E-03
G2BS3	150	80	3 μL	0.00E+00		0.00E+00	
G2TR1	100	80	3 μL	7.70E-02		2.05E-02	
G2TR2	100	80	3 μL	0.00E+00	2.84E-02	0.00E+00	7.56E-03
G2TR3	100	80	3 μL	8.09E-03		2.16E-03	
G2BR1	100	80	3 μL	1.82E-04		4.85E-05	
G2BR2	80	80	3 μL	8.31E-03	3.92E-03	2.77E-03	1.30E-03
G2BR3	80	80	3 μL	3.26E-03		1.09E-03	
G2TM1	3150	80	3 μL	2.13E-06		1.80E-08	
G2TM2	100	80	3 μL	2.02E-06	7.82E-06	5.39E-07	1.04E-06
G2TM3	200	80	3 μL	1.93E-05		2.57E-06	
G2BM1	170	80	3 μL	3.37E-06		5.29E-07	
G2BM2	280	80	3 μL	6.60E-06	4.99E-06	6.29E-07	5.79E-07
G2BM3	200	80	3 μL	N/A		N/A	
W7AS1	100	80	3 μL	2.24E-05		5.97E-06	
W7AS2	100	80	3 μL	1.46E-05	1.17E-04	3.89E-06	2.33E-05
W7AS3	140	80	3 μL	3.15E-04		6.00E-05	
W7BS1	50	80	3 μL	0.00E+00		0.00E+00	
W7BS2	50	80	3 μL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
W7BS3	40	80	3 μL	0.00E+00		0.00E+00	
W7AR1	40	80	3 μL	4.31E-05		2.87E-05	
W7AR2	80	80	3 μL	1.49E-05	2.24E-05	4.97E-06	1.22E-05
W7AR3	80	80	3 μL	9.08E-06		3.03E-06	
W7BR1	50	80	3 μL	0.00E+00		0.00E+00	
W7BR2	60	80	3 μL	0.00E+00	1.64E-01	0.00E+00	8.73E-02
W7BR3	50	80	3 μL	4.91E-01		2.62E-01	

www.manaraa.com

Table 3.19 (continued)

I7A1	40	80	3 μL	0.00E+00		0.00E+00	
I7A2	40	80	3 μL	0.00E+00	3.06E-06	0.00E+00	2.04E-06
I7A3	40	80	3 μL	9.17E-06		6.11E-06	
I7B1	60	80	3 μL	0.00E+00		0.00E+00	
I7B2	50	80	3 μL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
I7B3	50	80	3 μL	0.00E+00		0.00E+00	
V7A1	400	80	3 μL	2.84E-06		1.89E-07	
V7A2	400	80	3 μL	0.00E+00	9.47E-07	0.00E+00	6.31E-08
V7A3	400	80	3 μL	0.00E+00		0.00E+00	
V7B1	400	80	3 μL	0.00E+00		0.00E+00	
V7B2	400	80	3 μL	9.56E-06	7.62E-06	6.37E-07	6.07E-07
V7B3	300	80	3 μL	1.33E-05		1.18E-06	
W4AS1	80	80	3 μL	7.77E-03		2.59E-03	
W4AS2	90	80	3 μL	2.64E-02	2.00E-02	7.82E-03	3.77E-03
W4AS3	760	80	3 μL	2.59E-02		9.09E-04	
W4BS1	900	80	3 μL	2.72E-03		8.06E-05	
W4BS2	76	80	3 μL	1.21E-02	5.07E-03	4.25E-03	1.47E-03
W4BS3	130	80	3 μL	3.93E-04		8.06E-05	
W4BR1	150	80	3 μL	3.84E-05		6.83E-06	
W4AR2	90	80	3 μL	5.21E-05	4.33E-05	1.54E-05	1.24E-05
W4AR3	70	80	3 μL	3.94E-05		1.50E-05	
W4BR1	40	80	3 μL	1.10E-03		7.33E-04	
W4BR2	65	80	3 μL	3.37E-03	1.81E-03	1.38E-03	8.75E-04
W4BR3	50	80	3 μL	9.52E-04		5.08E-04	
W4AM1	60	80	3 μL	0.00E+00		0.00E+00	
W4AM2	50	80	3 μL	1.48E-05	6.93E-06	7.89E-06	3.96E-06
W4AM3	40	80	3 μL	5.98E-06		3.99E-06	
W4BM1	53	80	3 μL	3.24E-06		1.63E-06	
W4BM2	50	80	3 μL	3.45E-06	2.23E-06	1.84E-06	1.16E-06
W4BM3	66	80	3 μL	0.00E+00		0.00E+00	
G1AS1	120	80	3 μL	0.00E+00		0.00E+00	
G1AS2	170	80	3 μL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G1AS3	200	80	3 μL	0.00E+00		0.00E+00	
G1BS1	200	80	3 μL	0.00E+00		0.00E+00	
G1BS2	300	80	3 μL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G1BS3	500	80	3 μL	0.00E+00		0.00E+00	
G1AR1	62	80	3 μL	0.00E+00		0.00E+00	
G1AR2	75	80	3 μL	0.00E+00	3.43E-07	0.00E+00	9.16E-08

www.manaraa.com

Table 3.19 (continued)

G1AR3	100	80	3 µL	1.03E-06		2.75E-07	
G1BR1	97	80	3 µL	3.45E-06		9.48E-07	
G1BR2	100	80	3 µL	1.29E-05	8.57E-06	3.44E-06	2.02E-06
G1BR3	150	80	3 µL	9.37E-06		1.67E-06	
G1AM1	500	80	3 µL	3.76E-07		2.01E-08	
G1AM2	430	80	3 µL	2.81E-07	2.19E-07	1.74E-08	1.25E-08
G1AM3	427	80	3 µL	0.00E+00		0.00E+00	
G1BM1	450	80	3 µL	3.76E-06		2.23E-07	
G1BM2	360	80	3 µL	N/A	3.76E-06	N/A	2.23E-07
G1BM3	540	80	3 µL	N/A		N/A	
S1AF1	50	80	3 µL	1.03E-02		5.49E-03	
S1AF2	50	80	3 µL	2.37E-03	5.01E-03	1.26E-03	2.67E-03
S1AF3	50	80	3 µL	2.36E-03		1.26E-03	
S1AM1	50	80	3 µL	0.00E+00		0.00E+00	
S1AM2	50	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
S1AM3	60	80	3 µL	N/A		N/A	
S1AR1	50	80	3 µL	1.02E-03		5.44E-04	
S1AR2	50	80	3 µL	1.41E-04	4.09E-04	7.52E-05	2.18E-04
S1AR3	50	80	3 µL	6.65E-05		3.55E-05	
S2BF1	50	80	3 µL	9.87E-02		5.26E-02	
S2BF2	60	80	3 µL	0.00E+00	3.29E-02	0.00E+00	1.75E-02
S2BF3	50	80	3 µL	0.00E+00		0.00E+00	
S2BM1	60	80	3 µL	3.29E-05		1.46E-05	
S2BM2	50	80	3 µL	0.00E+00	1.73E-05	0.00E+00	7.69E-06
S2BM3	60	80	3 µL	1.90E-05		8.44E-06	
S2BR1	50	80	3 µL	0.00E+00		0.00E+00	
S2BR2	50	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
S2BR3	50	80	3 µL	0.00E+00		0.00E+00	
G3AF1	200	80	3 µL	0.00E+00		0.00E+00	
G3AF2	100	80	3 µL	0.00E+00	1.70E-07	0.00E+00	2.27E-08
G3AF3	200	80	3 µL	5.10E-07		6.80E-08	
G3AM1	200	80	3 µL	1.15E-06		1.53E-07	
G3AM2	200	80	3 µL	2.35E-06	2.34E-06	3.13E-07	3.12E-07
G3AM3	200	80	3 µL	3.51E-06		4.68E-07	
G3AR1	100	80	3 µL	8.53E-05		2.27E-05	
G3AR2	100	80	3 µL	6.00E-04	3.49E-04	1.60E-04	9.32E-05
G3AR3	100	80	3 µL	3.63E-04		9.68E-05	
G4BF1	100	80	3 µL	0.00E+00		0.00E+00	
G4BF2	200	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00

المنارة للاستشارات
www.manaraa.com

Table 3.19 (continued)

G4BF3	200	80	3 µL	0.00E+00		0.00E+00	
G4BM1	300	80	3 µL	0.00E+00		0.00E+00	
G4BM2	200	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G4BM3	200	80	3 µL	0.00E+00		0.00E+00	
G4BR1	100	80	3 µL	0.00E+00		0.00E+00	
G4BR2	100	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G4BR3	100	80	3 µL	0.00E+00		0.00E+00	

The wet building materials were analyzed for the presence of *Chaetomium globosum* and this data are shown in Figure 3.45.

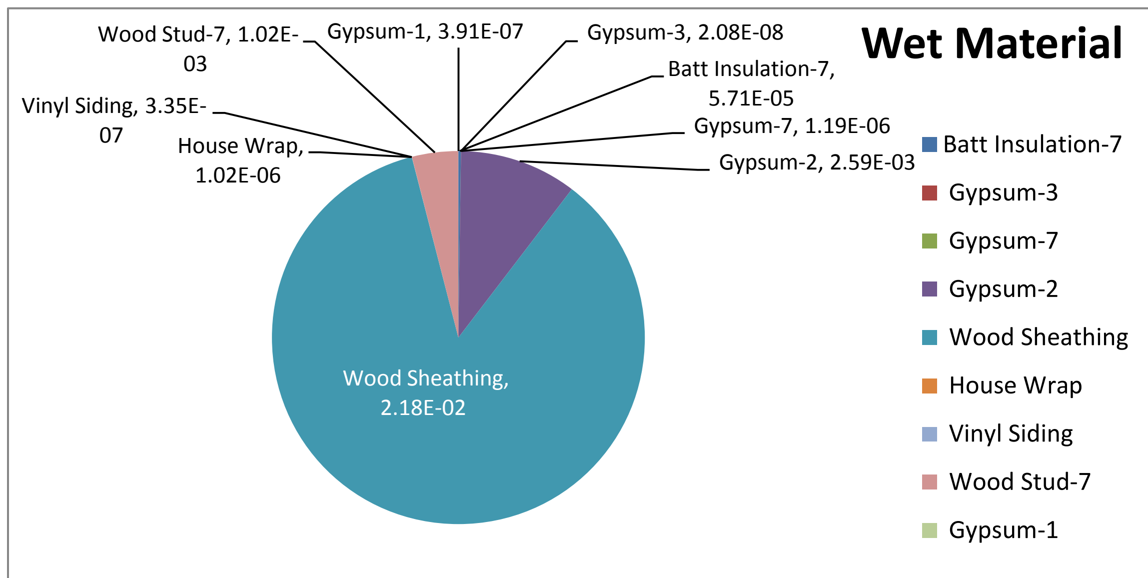

Figure 3.45 The distribution of *Chaetomium globosum*, based on averaged DNA concentrations, on the different wet building materials. Samples with a 7 came from the same wall unit.

The DNA averages plotted in Figure 3.45 shows that *Chaetomium globosum* was present in all materials. The wood sheathing contained high concentrations of DNA

المنارة للاستشارات
www.manaraa.com

($2.18E^{-02}$), followed by gypsum-2 ($2.59E^{-03}$) and the wood stud-7 ($1.02E^{-03}$). The batt insulation (5.71E-05), house wrap (1.02E-06), and gypsum-7 (1.19E-06) supported moderate levels of *C. globosum*, while in the all other materials the concentration was quite low. There were no statistical differences found in *Chaetomium globosum* concentrations among the different wet materials (Table 3.20).

Table 3.20 Statistical (ANOVA) anaylsis of the significant difference of wet building materials. (Means with the same letter are not significantly different).

ANOVA Statistical Differences			
Tukey Grouping	**Mean**	**N**	**Material**
A	0.021831	12	Wood sheathing
A	0.002737	17	Gypsum-2
A	0.001022	18	Wood stud
A	0.000057	18	Batt Insulation
A	0.000001	18	Gypsum-7
A	0.000001	6	House wrap
A	0.000000	16	Gypsum-1
A	0.000000	6	Vinyl siding
A	0.000000	18	Gypsum-3

The presence of *Chaetomium globosum* was examined on individual components of the different wall materials and given in Figures 3.46-3.52.

المنارة للاستشارات
www.manaraa.com

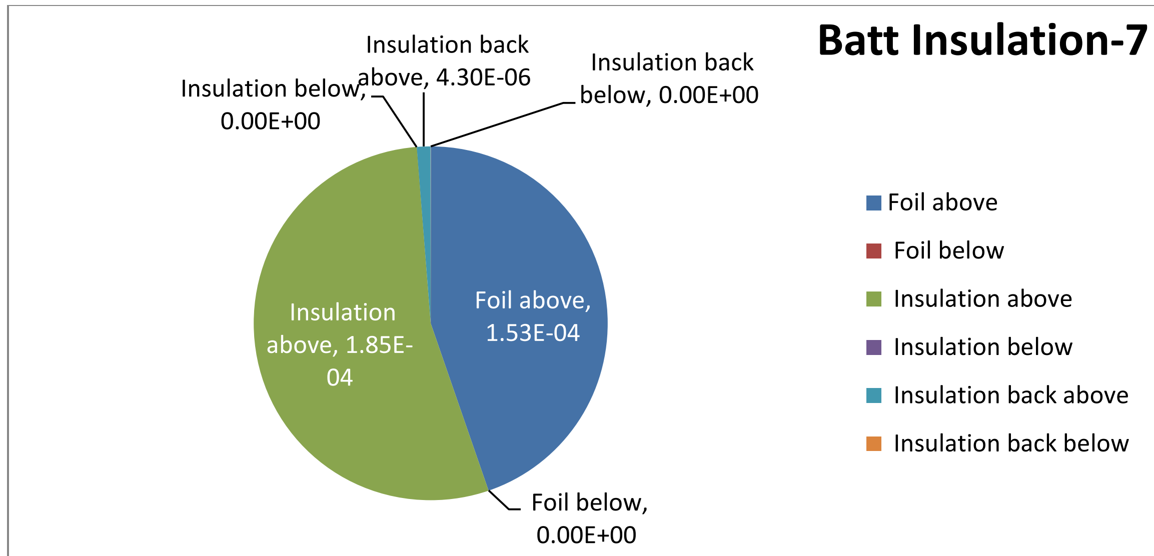

Figure 3.46 The distribution of *Chaetomium globosum*, based on DNA concentrations, on the different components of the wet batt insulation-7.

The distribution of *Chaetomium globosum* in the batt insulation (Figure 3.46) indicates that *Chaetomium globosum* was found in the highest concentration of $1.85E^{-04}$ above the water line on the insulation itself (green), followed closely by the foil above the water line ($1.53E^{-04}$). The back part of the insulation above the water line also contained moderately low levels of *C. globosum* at $4.30E^{-06}$. It is worth noting that *Chaetomium globosum* was found on the batt insulation only above the water line and not below the water line.

المنارة للاستشارات
www.manaraa.com

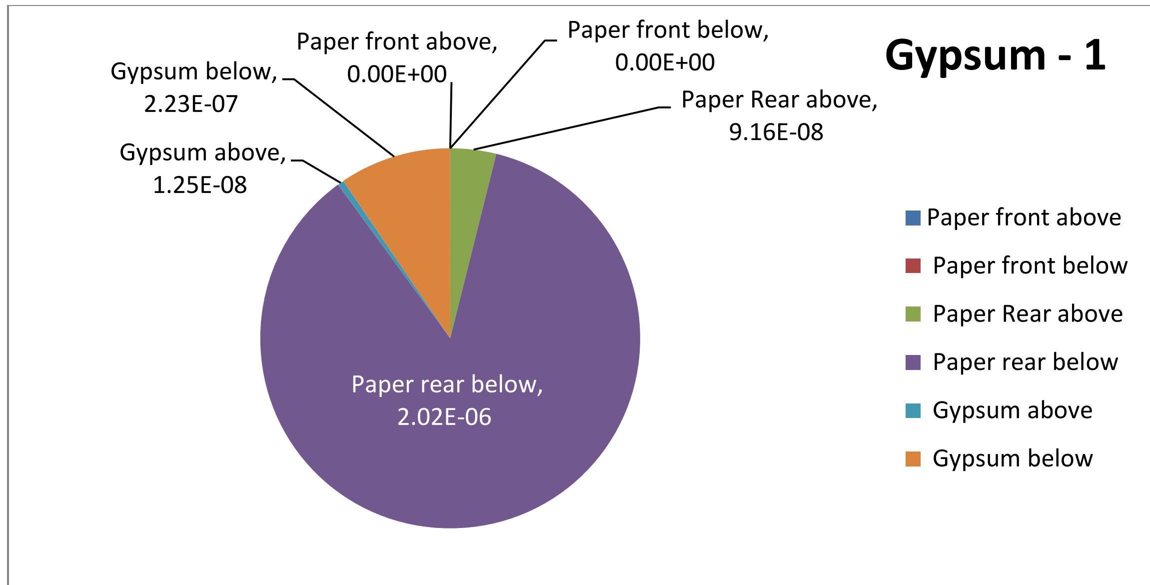

Figure 3.47 The distribution of *Chaetomium globosum*, based on DNA concentrations, on the different components of the wet gypsum-1.

Chaetomium globosum was found in moderate concentrations ($2.02E^{-06}$) only on the rear paper of gypsum-1 (Figure 3.47). All other material components of gypsum-1 contained very low to no detectable levels of *C. globosum*.

المنارة للاستشارات
www.manaraa.com

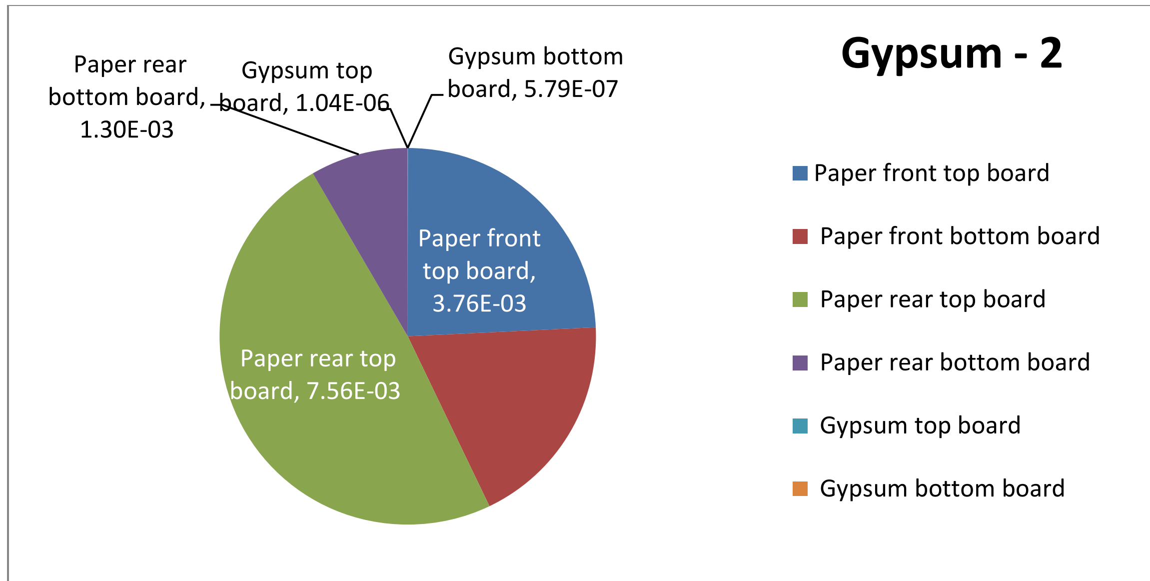

Figure 3.48 The distribution of *Chaetomium globosum*, based on DNA concentrations, on the different components of the wet gypsum-2.

For gypsum-2 (Figure 3.48) *Chaetomium globosum* was detected in moderately high concentrations on all paper components including the rear paper top of the board ($7.56E^{-03}$), the rear paper bottom ($1.30E^{-03}$), the paper front top of the board ($3.76E^{-03}$), and paper front bottom ($2.89E^{-03}$). The gypsum material itself contained low levels of this fungus.

www.manaraa.com

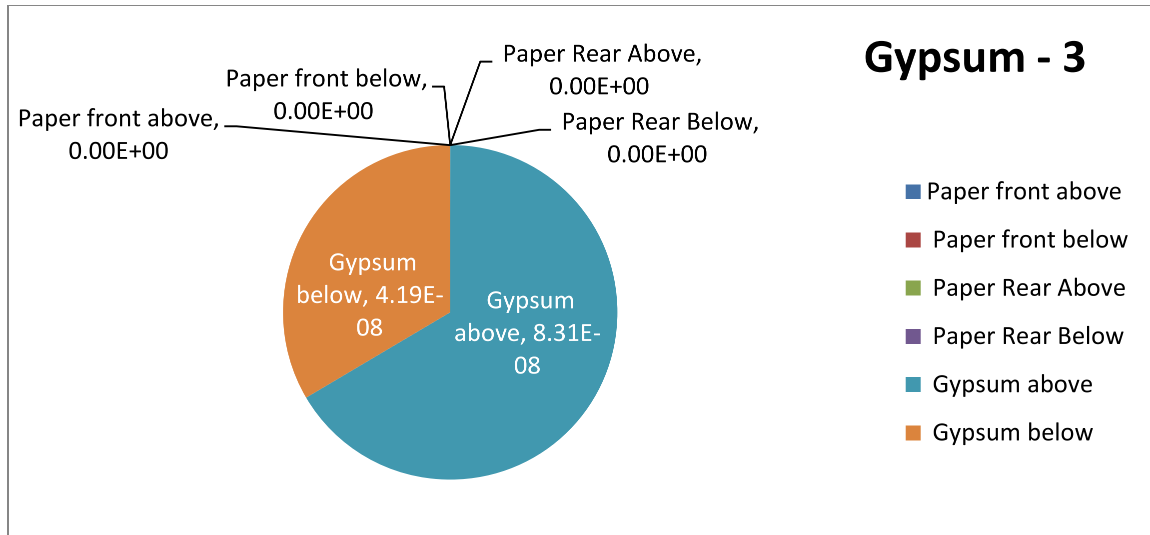

Figure 3.49 The distribution of *Chaetomium globosum*, based on DNA concentrations, on the different components of the wet gypsum-3 material.

Gypsum -3 in Figure 3.49 supported *Chaetomium globosum* only at very low levels on the gypsum above (8.31E^{-08}) and below (4.19E^{-08}) the water line. None of the paper components contained any detectable *Chaetomium globosum*.

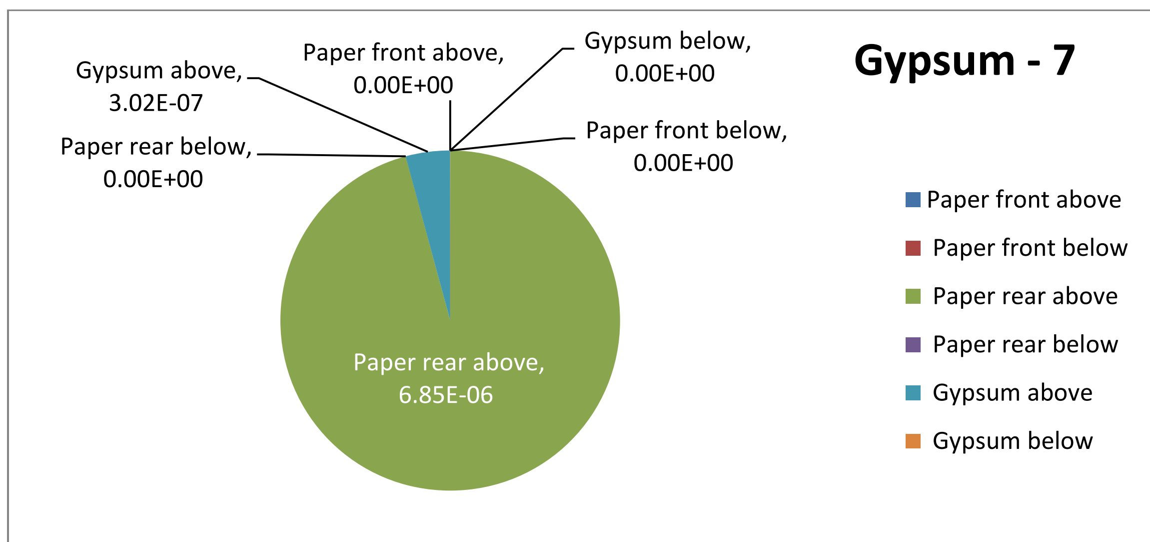

Figure 3.50 The distribution of *Chaetomium globosum*, based on DNA concentrations, on the different components of the wet gypsum-7.

www.manaraa.com

Chaetomium globosum was detected primarily on the paper rear above the water line in low concentrations (6.85E^{-06}) on the gypsum-7 wallboard (Figure 3.50). All other components of this material contained no or low *C. globosum* DNA.

Figure 3.51 The distribution of *Chaetomium globosum*, based on DNA concentrations, on the different components of the wet wood stud-7.

Chaetomium globosum was detected in all samples of the wood stud (Figure 3.51). This species was present in moderately high concentrations on the front section of the 2x4 wood stud above the water line (3.77E^{-03}) followed by the front section below the water line (1.47E^{-03}), and the rear of the wood stud below the water line (8.75E^{-04}). The wood stud rear above (1.24E^{-05}), wood stud middle above (3.96E^{-06}), and below (1.16E^{-06}) the water line supported very low concentrations of *Chaetomium globosum*.

المنارة للاستشارات
www.manaraa.com

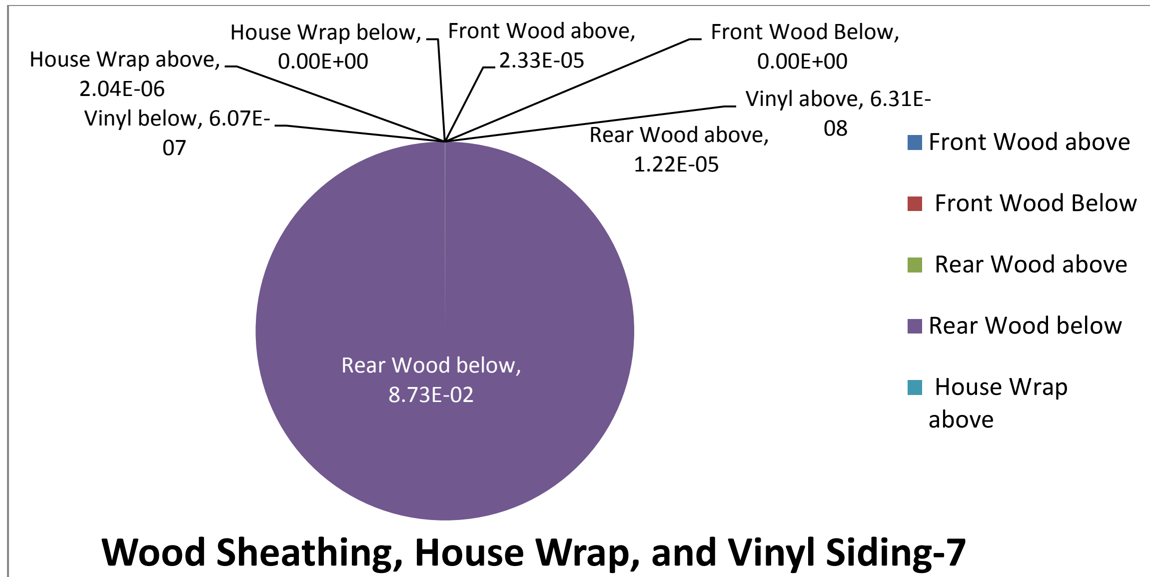

Figure 3.52 The distribution of *Chaetomium globosum*, based on DNA concentrations, on the wet wood sheathing, plus the house wrap, and vinyl siding-7 materials.

Figure 3.52 shows that *Chaetomium globosum* was found in high concentrations on the rear section of the wood sheathing below the water line ($8.73E^{-02}$). The front of the wood sheathing above the water line ($2.33E^{-05}$), the rear of the wood sheathing above the water line ($1.22E^{-05}$), and the house wrap above the water line ($2.04E^{-06}$) supported moderate-low concentrations of *C. globosum*. All other material components contained very low to no presence of this fungus. Statistics were performed for the wet materials on both above and below the water line for *C. globosum* and presented in Table 3.21.

المنارة للاستشارات
www.manaraa.com

Table 3.21 Statistical analysis on *Chaetomium globosum* both above and below the water line on all wet materials.

State	Waterline	Samples	Mean	Std Dev
wet	above	57	0.00022	0.001089
wet	below	57	0.004889	0.035298

Table 3.21 shows that *Chaetomium globosum* was present in higher concentrations above the water line than below the water line on the wet materials. The dry samples were examined for *Chaetomium globosum* on the wood stud and gypsum wallboard. The different dry materials examined for this mold species both above and below the water line are shown in Figure 3.53.

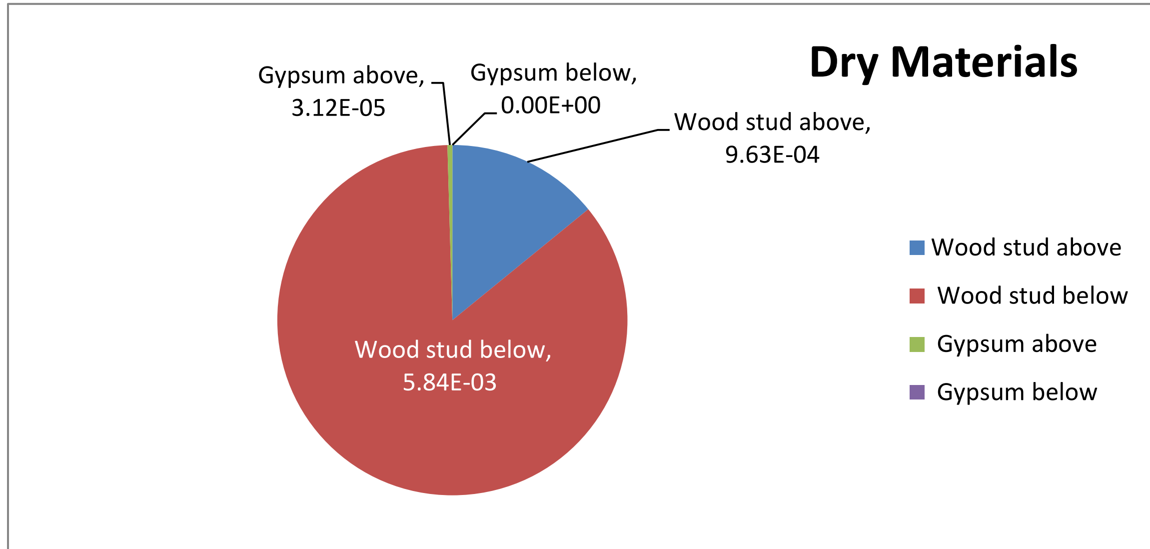

Figure 3.53 The distribution of *Chaetomium globosum*, based on DNA concentrations, on the different the dry building materials.

Chaetomium globosum was primarily found on the wood stud both below (5.84E^{-03}) and above (9.63E^{-04}) the water line as shown in Figure 3.53. Gypsum supported less of

www.manaraa.com

this species above the water line ($3.12E^{-05}$) and non-detectable below the water line. There were no statistical differences found in *C. globosum* concentrations among the different dry materials (Table 3.22).

Table 3.22 Statistical (ANOVA) anaylsis of the significant difference of dry building materials. (Means with the same letter are not significantly different).

ANOVA Statistical Difference			
Tukey Grouping	**Mean**	**N**	**Material**
A	0.003608	17	Wood stud
A	0.000016	18	Gypsum

The concentrations of *Chaetomium globosum* were examined on individual components of the materials and this data are graphed in Figures 3.54- 3.55.

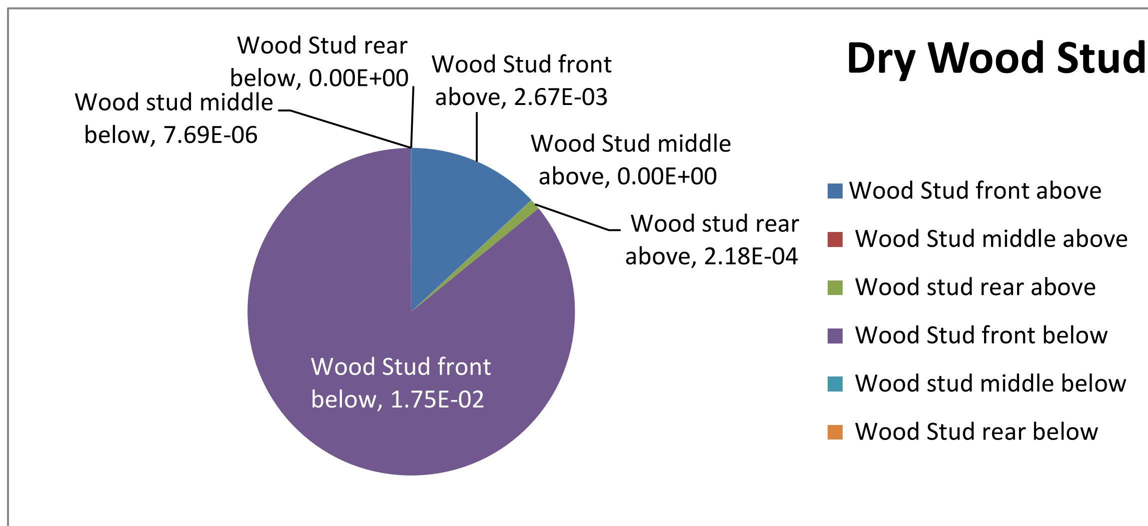

Figure 3.54 The distribution of *Chaetomium globosum*, based on DNA concentrations, on the different components of the dry wood stud.

www.manaraa.com

Figure 3.54 shows that *Chaetomium globosum* was present in a high concentration of 1.75E^{-02} on the front section of the 2x4 wood stud below the water line. The species was also found in moderately high levels on the front section of the wood stud above the water line (2.67E^{-03}), and to a lesser extent on the rear section of the wood stud above the water line (2.18E^{-04}). The other components of the wood stud supported much lower to no concentrations of *C. globosum*.

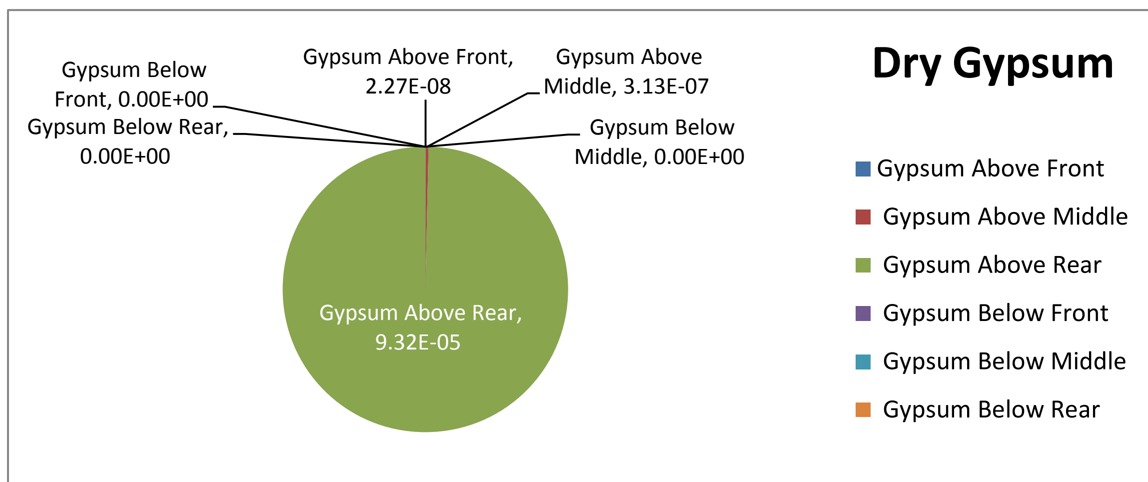

Figure 3.55 The distribution of *Chaetomium globosum*, based on DNA concentrations, on the different components of the dry gypsum.

Figure 3.55 shows that *Chaetomium globosum* was found in moderate concentrations on the paper rear of the gypsum wallboard above the water line (9.32E^{-05}). The other wall materials contained very low to no *Chaetomium globosum*.

Whenever *Chaetomium globosum* was detected in notably high concentrations (E^{-02}-E-^{03}), it was always on a cellulose-based material. This included the wet wood sheathing (2.18E^{-02}), the wet paper front and back of gypsum-2 (2.59E^{-03}), the wet wood stud (1.02E^{-03}) and the dry wood stud (1.75E^{-02}). The non- cellulose materials did support

المنارة للاستشارات
www.manaraa.com

the presence of *C. globosum* but at much reduced level. *C. globosum* was likely to occur 20x more frequently below the water line compared to above the water line. The high concentration of this species on the dry wood stud seven months after flooding is notable.

Paecilomyces variotii

Paecilomyces variotii can occur worldwide and is a common air-borne contaminant. The colonies of *Paecilomyces variotii* grow rapidly and consist of a dense felt of numerous conidiophores appearing powdery and yellowish-brown. *Paecilomyces variotii* can grow on damp walls, wet plaster work, and in carpet dust (Samson et al. 2010). This type of fungi causes invasive mycoses in compromised individuals and has been reported to be resistant against preservatives such as sorbic, benzoic, and propionic acid. The toxins that can be produced are viriditoxin and patulin (Flannigan et al. 2011). The DNA concentrations of *Paecilomyces variotii* was determined using real-time PCR of each wall construction material and converted to the amount of fungal DNA in nannograms per weight of the material sample (Table 3.23).

Table 3.23 Real-time PCR results of *Paecilomyces variotii* DNA in nannograms per weight of material

Sample Code	Material Weight (mg)	Extraction volume	Amount µL used	Real Time (ng)	Average Reps	(ng)DNA/(mg) Material	Average DNA/Material
B1AP1	100	80	3 µL	7.52E-04		2.01E-04	
B1AP2	100	80	3 µL	6.32E-04	5.31E-04	1.69E-04	1.42E-04
B1AP3	100	80	3 µL	2.08E-04		5.55E-05	
B1BP1	140	80	3 µL	0.00E+00		0.00E+00	
B1BP2	140	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
B1BP3	140	80	3 µL	0.00E+00		0.00E+00	

www.manaraa.com

Table 3.23 (continued)

B1AN1	50	80	3 μL	1.37E-03		7.31E-04	
B1AN2	50	80	3 μL	0.00E+00	4.57E-04	0.00E+00	2.44E-04
B1AN3	50	80	3 μL	1.13E-06		6.03E-07	
B1BN1	100	80	3 μL	1.62E-04		4.32E-05	
B1BN2	100	80	3 μL	3.72E-04	2.29E-04	9.92E-05	6.11E-05
B1BN3	100	80	3 μL	1.53E-04		4.08E-05	
B1AF1	50	80	3 μL	0.00E+00		0.00E+00	
B1AF2	50	80	3 μL	2.38E-07	2.77E-04	1.27E-07	1.48E-04
B1AF3	50	80	3 μL	8.32E-04		4.44E-04	
B1BF1	100	80	3 μL	0.00E+00		0.00E+00	
B1BF2	100	80	3 μL	3.41E-05	1.31E-05	9.09E-06	3.50E-06
B1BF3	100	80	3 μL	5.33E-06		1.42E-06	
G3AS1	200	80	3 μL	0.00E+00		0.00E+00	
G3AS2	200	80	3 μL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G3AS3	200	80	3 μL	0.00E+00		0.00E+00	
G3BS1	250	80	3 μL	0.00E+00		0.00E+00	
G3BS2	250	80	3 μL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G3BS3	250	80	3 μL	0.00E+00		0.00E+00	
G3AR1	250	80	3 μL	0.00E+00		0.00E+00	
G3AR2	250	80	3 μL	5.77E-07	1.92E-07	6.15E-08	2.05E-08
G3AR3	250	80	3 μL	0.00E+00		0.00E+00	
G3BR1	300	80	3 μL	0.00E+00		0.00E+00	
G3BR2	300	80	3 μL	1.28E-07	5.20E-05	1.14E-08	4.63E-06
G3BR3	300	80	3 μL	1.56E-04		1.39E-05	
G3AM1	500	80	3 μL	0.00E+00		0.00E+00	
G3AM2	500	80	3 μL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G3AM3	500	80	3 μL	0.00E+00		0.00E+00	
G3BM1	500	80	3 μL	1.71E-05		9.12E-07	
G3BM2	500	80	3 μL	7.85E-06	8.32E-06	4.19E-07	4.44E-07
G3BM3	500	80	3 μL	0.00E+00		0.00E+00	
G4BS1	210	80	3 μL	0.00E+00		0.00E+00	
G4BS2	200	80	3 μL	3.20E-04	2.67E-04	4.27E-05	6.40E-05
G4BS3	200	80	3 μL	4.80E-04		6.40E-05	
G4AR1	100	80	3 μL	1.12E-04		2.99E-05	
G4AR2	100	80	3 μL	0.00E+00	2.97E-04	0.00E+00	7.92E-05
G4AR3	100	80	3 μL	7.79E-04		2.08E-04	
G4BR1	200	80	3 μL	1.79E-04		2.39E-05	
G4BR2	200	80	3 μL	8.47E-07	5.99E-05	1.13E-07	7.99E-06

للاستشارات المنارة
www.manaraa.com

Table 3.23 (continued)

G4BR3	200	80	3 µL	0.00E+00		0.00E+00	
G4AM1	300	80	3 µL	0.00E+00		0.00E+00	
G4AM2	300	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G4AM3	320	80	3 µL	0.00E+00		0.00E+00	
G4BM1	500	80	3 µL	0.00E+00		0.00E+00	
G4BM2	520	80	3 µL	3.17E-04	1.67E-04	1.63E-05	8.69E-06
G4BM3	500	80	3 µL	1.84E-04		9.81E-06	
G2TS1	150	80	3 µL	0.00E+00		0.00E+00	
G2TS2	160	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G2TS3	150	80	3 µL	0.00E+00		0.00E+00	
G2BS1	160	80	3 µL	0.00E+00		0.00E+00	
G2BS2	150	80	3 µL	0.00E+00	4.83E-05	0.00E+00	8.59E-06
G2BS3	150	80	3 µL	1.45E-04		2.58E-05	
G2TR1	100	80	3 µL	0.00E+00		0.00E+00	
G2TR2	100	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G2TR3	100	80	3 µL	0.00E+00		0.00E+00	
G2BR1	100	80	3 µL	0.00E+00		0.00E+00	
G2BR2	80	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G2BR3	80	80	3 µL	0.00E+00		0.00E+00	
G2TM1	3150	80	3 µL	4.54E-02		3.84E-04	
G2TM2	100	80	3 µL	0.00E+00	1.51E-02	0.00E+00	1.28E-04
G2TM3	200	80	3 µL	0.00E+00		0.00E+00	
G2BM1	170	80	3 µL	0.00E+00		0.00E+00	
G2BM2	280	80	3 µL	0.00E+00	6.30E-05	0.00E+00	8.40E-06
G2BM3	200	80	3 µL	1.89E-04		2.52E-05	
W7AS1	100	80	3 µL	0.00E+00		0.00E+00	
W7AS2	100	80	3 µL	1.47E-06	4.90E-07	3.92E-07	1.31E-07
W7AS3	140	80	3 µL	0.00E+00		0.00E+00	
W7BS1	50	80	3 µL	5.78E-05		3.08E-05	
W7BS2	50	80	3 µL	1.00E-04	8.79E-05	5.33E-05	5.16E-05
W7BS3	40	80	3 µL	1.06E-04		7.07E-05	
W7AR1	40	80	3 µL	8.32E-07		5.55E-07	
W7AR2	80	80	3 µL	0.00E+00	2.77E-07	0.00E+00	1.85E-07
W7AR3	80	80	3 µL	0.00E+00		0.00E+00	
W7BR1	50	80	3 µL	0.00E+00		0.00E+00	
W7BR2	60	80	3 µL	2.35E-04	2.78E-04	1.04E-04	5.77E-05
W7BR3	50	80	3 µL	1.29E-04		6.88E-05	
I7A1	40	80	3 µL	0.00E+00		0.00E+00	

المنارة للاستشارات
www.manaraa.com

Table 3.23 (continued)

I7A2	40	80	3 µL	0.00E+00	7.33E-07	0.00E+00	4.89E-07
I7A3	40	80	3 µL	2.20E-06		1.47E-06	
I7B1	60	80	3 µL	1.26E-04		5.60E-05	
I7B2	50	80	3 µL	2.65E-05	7.96E-05	1.41E-05	3.87E-05
I7B3	50	80	3 µL	8.63E-05		4.60E-05	
V7A1	400	80	3 µL	0.00E+00		0.00E+00	
V7A2	400	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
V7A3	400	80	3 µL	0.00E+00		0.00E+00	
V7B1	400	80	3 µL	6.34E-06		4.23E-07	
V7B2	400	80	3 µL	1.16E-04	4.20E-05	7.73E-06	2.83E-06
V7B3	300	80	3 µL	3.67E-06		3.26E-07	
W4AS1	80	80	3 µL	1.34E-01		4.47E-02	
W4AS2	90	80	3 µL	1.67E-01	1.31E-01	4.95E-02	3.24E-02
W4AS3	760	80	3 µL	9.09E-02		3.19E-03	
W4BS1	900	80	3 µL	2.93E-04		8.68E-06	
W4BS2	76	80	3 µL	3.67E-04	2.20E-04	1.29E-04	4.59E-05
W4BS3	130	80	3 µL	5.97E-07		1.22E-07	
W4BR1	150	80	3 µL	1.73E-03		3.08E-04	
W4AR2	90	80	3 µL	9.63E-03	1.86E-02	2.85E-03	6.69E-03
W4AR3	70	80	3 µL	4.44E-02		1.69E-02	
W4BR1	40	80	3 µL	2.48E-03		1.65E-03	
W4BR2	65	80	3 µL	1.90E-03	2.40E-03	7.79E-04	1.31E-03
W4BR3	50	80	3 µL	2.82E-03		1.50E-03	
W4AM1	60	80	3 µL	1.49E-02		6.62E-03	
W4AM2	50	80	3 µL	1.51E-02	1.20E-02	8.05E-03	6.21E-03
W4AM3	40	80	3 µL	5.93E-03		3.95E-03	
W4BM1	53	80	3 µL	6.53E-05		3.29E-05	
W4BM2	50	80	3 µL	2.21E-04	2.18E-04	1.18E-04	9.99E-05
W4BM3	66	80	3 µL	3.69E-04		1.49E-04	
G1AS1	120	80	3 µL	0.00E+00		0.00E+00	
G1AS2	170	80	3 µL	1.12E-04	3.75E-05	1.76E-05	5.87E-06
G1AS3	200	80	3 µL	4.05E-07		5.40E-08	
G1BS1	200	80	3 µL	3.97E-06		5.29E-07	
G1BS2	300	80	3 µL	0.00E+00	1.32E-06	0.00E+00	1.76E-07
G1BS3	500	80	3 µL	0.00E+00		0.00E+00	
G1AR1	62	80	3 µL	0.00E+00		0.00E+00	
G1AR2	75	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G1AR3	100	80	3 µL	0.00E+00		0.00E+00	

المنارة للاستشارات
www.manaraa.com

Table 3.23 (continued)

G1BR1	97	80	3 µL	0.00E+00		0.00E+00	
G1BR2	100	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G1BR3	150	80	3 µL	0.00E+00		0.00E+00	
G1AM1	500	80	3 µL	0.00E+00		0.00E+00	
G1AM2	430	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G1AM3	427	80	3 µL	0.00E+00		0.00E+00	
G1BM1	450	80	3 µL	2.77E-05		1.64E-06	
G1BM2	360	80	3 µL	0.00E+00	9.23E-06	0.00E+00	5.47E-07
G1BM3	540	80	3 µL	0.00E+00		0.00E+00	
S1AF1	50	80	3 µL	6.70E-03		3.57E-03	
S1AF2	50	80	3 µL	1.56E-05	2.24E-03	8.32E-06	1.20E-03
S1AF3	50	80	3 µL	1.65E-05		8.80E-06	
S1AM1	50	80	3 µL	5.99E-02		3.19E-02	
S1AM2	50	80	3 µL	0.00E+00	1.49E-01	0.00E+00	6.78E-02
S1AM3	60	80	3 µL	3.86E-01		1.72E-01	
S1AR1	50	80	3 µL	1.56E-02		8.32E-03	
S1AR2	50	80	3 µL	1.67E-02	1.08E-02	8.91E-03	5.74E-03
S1AR3	50	80	3 µL	0.00E+00		0.00E+00	
S2BF1	50	80	3 µL	2.53E-04		1.35E-04	
S2BF2	60	80	3 µL	3.49E-04	2.81E-04	1.55E-04	1.40E-04
S2BF3	50	80	3 µL	2.42E-04		1.29E-04	
S2BM1	60	80	3 µL	0.00E+00		0.00E+00	
S2BM2	50	80	3 µL	5.77E-05	1.92E-05	3.08E-05	1.03E-05
S2BM3	60	80	3 µL	0.00E+00		0.00E+00	
S2BR1	50	80	3 µL	2.87E-04		1.53E-04	
S2BR2	50	80	3 µL	2.75E-04	3.84E-04	1.47E-04	2.05E-04
S2BR3	50	80	3 µL	5.89E-04		3.14E-04	
G3AF1	200	80	3 µL	1.76E-04		2.35E-05	
G3AF2	100	80	3 µL	0.00E+00	1.43E-04	0.00E+00	1.91E-05
G3AF3	200	80	3 µL	2.53E-04		3.37E-05	
G3AM1	200	80	3 µL	5.99E-04		7.99E-05	
G3AM2	200	80	3 µL	2.82E-04	3.37E-04	3.76E-05	4.50E-05
G3AM3	200	80	3 µL	1.31E-04		1.75E-05	
G3AR1	100	80	3 µL	3.44E-06		9.17E-07	
G3AR2	100	80	3 µL	4.10E-04	1.39E-04	1.09E-04	3.70E-05
G3AR3	100	80	3 µL	3.19E-06		8.51E-07	
G4BF1	100	80	3 µL	5.21E-04		1.39E-04	
G4BF2	200	80	3 µL	1.34E-04	2.89E-04	1.79E-05	6.17E-05
G4BF3	200	80	3 µL	2.13E-04		2.84E-05	
G4BM1	300	80	3 µL	0.00E+00		0.00E+00	

المنارة للاستشارات
www.manaraa.com

Table 3.23 (continued)

G4BM2	200	80	3 µL	1.00E-04	3.33E-05	1.33E-05	4.44E-06
G4BM3	200	80	3 µL	0.00E+00		0.00E+00	
G4BR1	100	80	3 µL	1.31E-05		3.49E-06	
G4BR2	100	80	3 µL	5.54E-05	5.38E-05	1.48E-05	1.44E-05
G4BR3	100	80	3 µL	9.30E-05		2.48E-05	

The wet building material was analyzed for *Paecilomyces variotii* on each type of wet material and this data are shown in Figure 3.56.

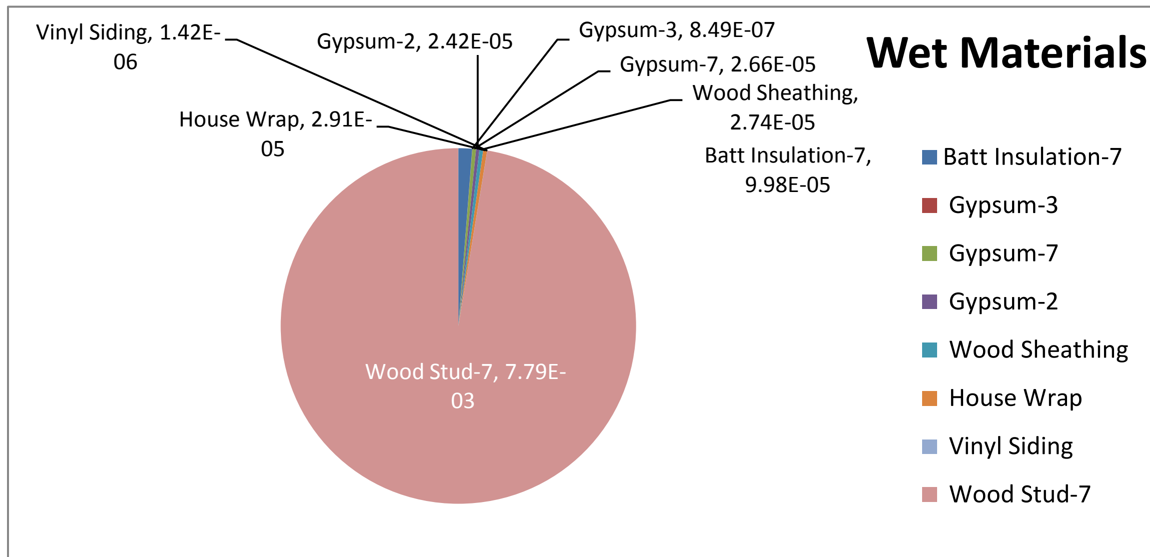

Figure 3.56 The distribution of *Paecilomyces variotii*, based on averaged DNA concentrations, on the different wet building materials. Samples with a 7 came from the same wall unit.

The wet material averages in Figure 3.56 show *Paecilomyces variotii* was present on all materials. The wood stud contained the highest concentration at 7.79E^{-03}, followed by gypsum-2 (2.42E^{-05}), gypsum-7(2.66E^{-05}), and wood sheathing (2.74E^{-05}). The other components supported only low concentrations. The concentration of *Paecilomyces*

المنارة للاستشارات
www.manaraa.com

variotii on the wood stud was statistically greater than on any of the other wall materials (Table 3.24).

Table 3.24 Statistical (ANOVA) analysis of the significant difference of dry building materials. (Means with the same letter are not significantly different).

ANOVA Statistical Difference			
Tukey Grouping	**Mean**	**N**	**Material**
A	0.007801	18	Wood stud
B	0.000100	18	Batt Insulation
B	0.000027	12	Wood sheathing
B	0.000024	18	Gypsum-2
B	0.000022	18	Gypsum-7
B	0.000020	6	House wrap
B	0.000001	6	Vinyl Siding
B	0.000001	18	Gypsum-1
B	0.000001	18	Gypsum-3

The distributions of *Paecilomyces varitotii* on individual components of each material are given in Figures 3.57-3.63.

المنارة للاستشارات

www.manaraa.com

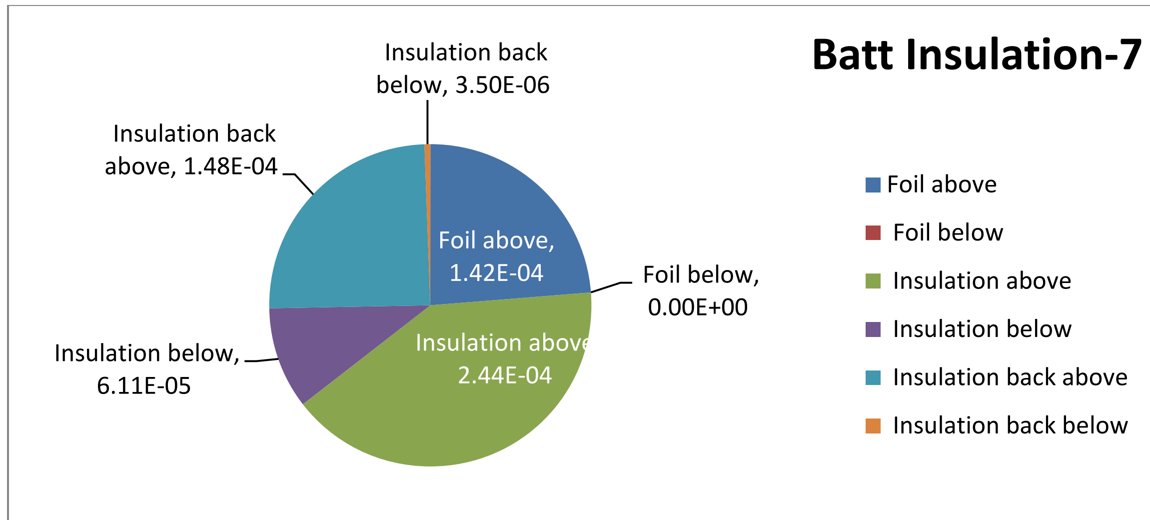

Figure 3.57 The distribution of *Paecilomyces variotii*, based on DNA concentrations, on the different components of batt insulation-7.

The distribution of *Paecilomyces variotii* in the batt insulation (Figure 3.57) indicates that this species was found in the highest concentration above the water line on the insulation itself (green) at $2.44E^{-04}$ followed by the the back of the insulation ($1.48E^{-04}$), and the foil ($1.42E^{-04}$). The insulation below the water line ($6.11E^{-05}$ and $3.50E^{-06}$) contained moderately low levels of *P. variotii*.

www.manaraa.com

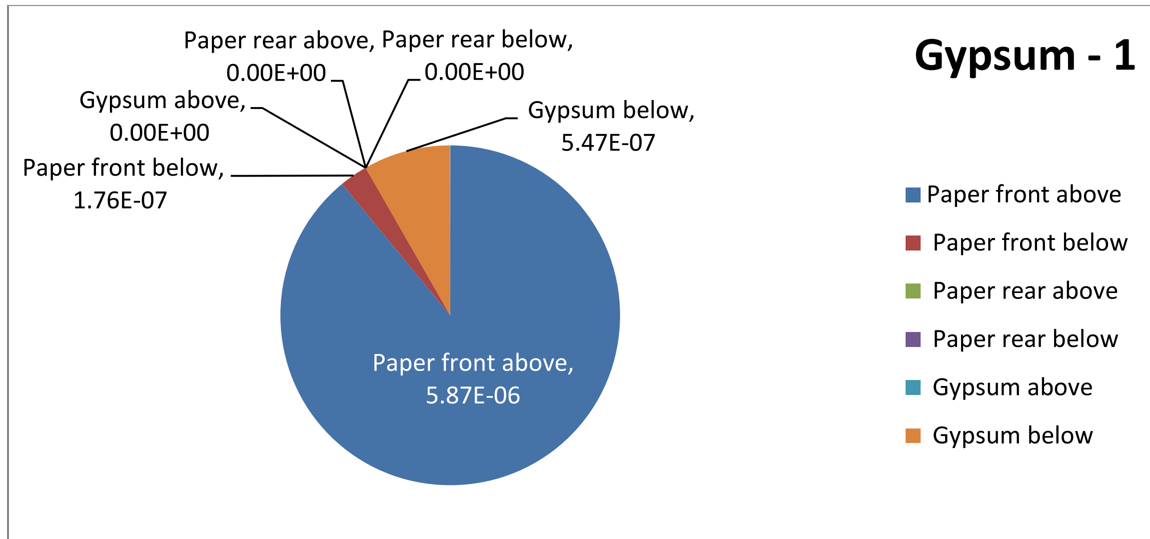

Figure 3.58 The distribution of *Paecilomyces variotii*, based on DNA concentrations, on the different components of the wet gypsum-1.

Figure 3.58 shows that on the gypsum-1 board *Paecilomyces variotii* was found at a concentration of $5.87E^{-06}$ above the water line on the paper front. *P. variotii* was also found on the gypsum itself and the front of the paper however, the concentrations were very low (E^{-07}).

المنارة للاستشارات
www.manaraa.com

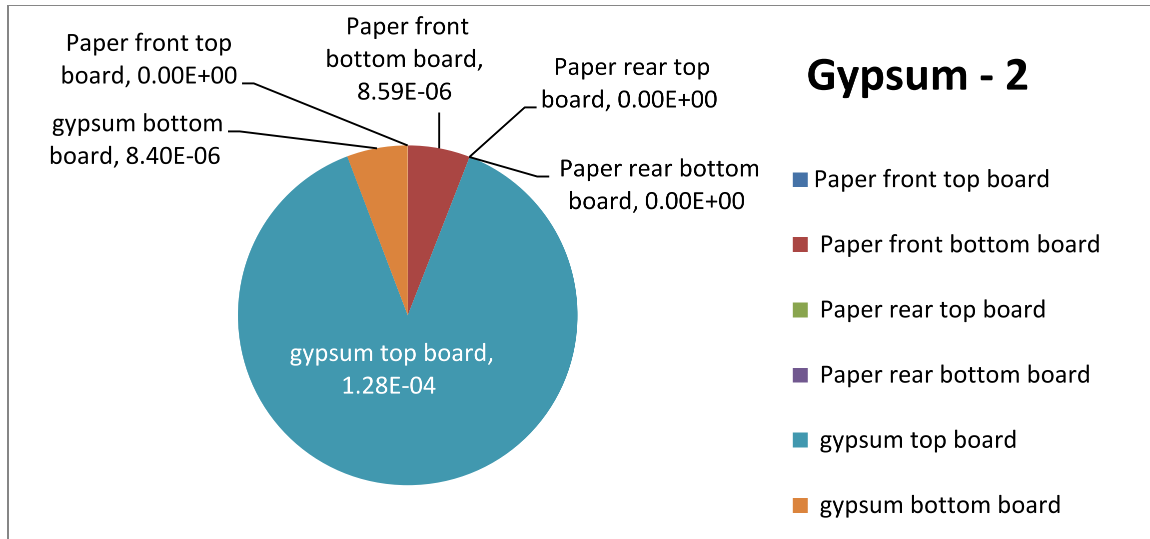

Figure 3.59 The distribution of *Paecilomyces variotii*, based on DNA concentrations, on the different components of the wet gypsum-2.

Gypsum -2 in Figure 3.59 shows that *Paecilomyces variotii* was found in moderate concentrations in the gypsum at the top of the board ($1.28E^{-04}$). The other components that contained *P. variotii* included the paper front bottom of the board ($8.59E^{-06}$) and the gypsum bottom of the board ($8.40E^{-06}$).

المنارة للاستشارات
www.manaraa.com

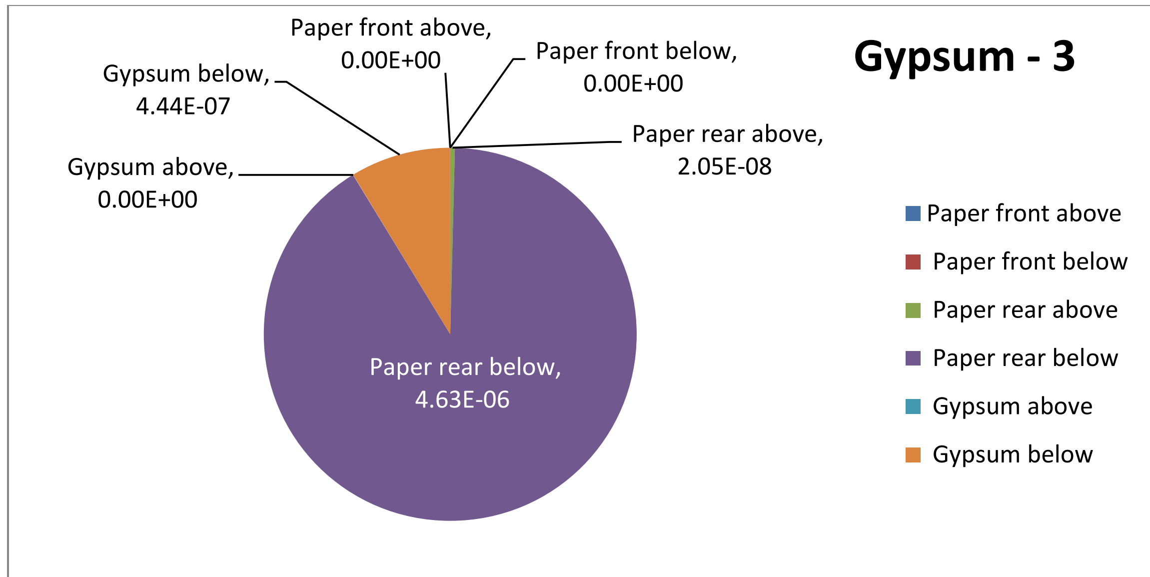

Figure 3.60 The distribution of *Paecilomyces variotii*, based on DNA concentrations, on the different components of the wet gypsum-3.

Paecilomyces variotii was primarily located in moderately low concentrations on the rear paper below the water line ($4.63E^{-06}$) in Figure 3.60. The other components that contained *P. variotii* in low concentrations included the gypsum itself below the water line ($4.44E^{-07}$) and the rear paper above the water line ($2.05E^{-08}$).

المنارة للاستشارات
www.manaraa.com

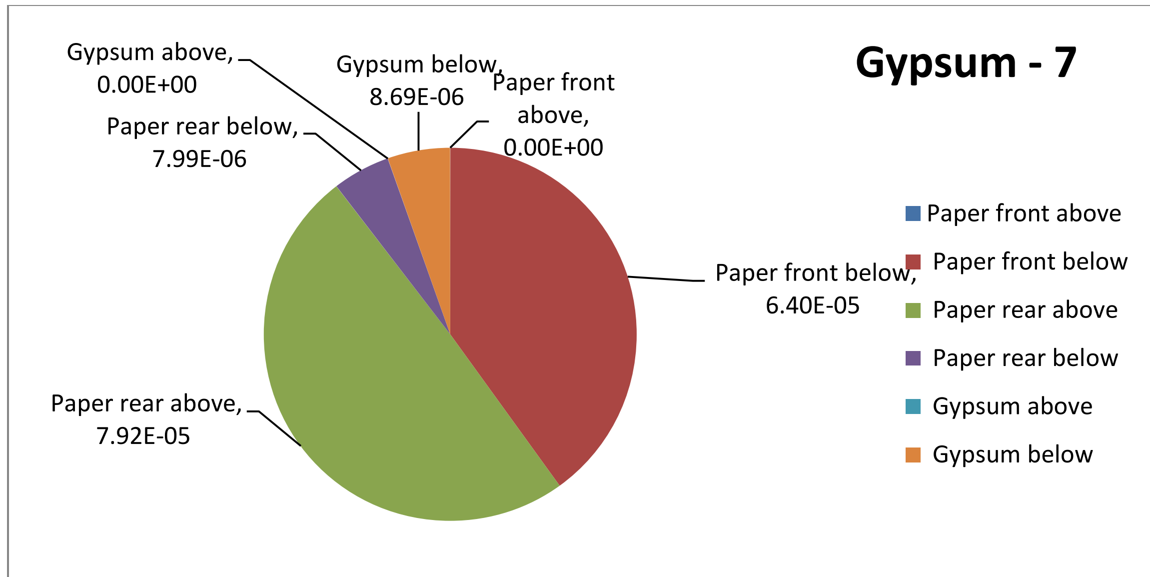

Figure 3.61 The distribution of *Paecilomyces variotii*, based on DNA concentrations, on the different components of the wet gypsum-7.

Figure 3.61 shows that *Paecilomyces variotii* was present in the gypsum-7 board at a concentration of 7.92E^{-05} on the rear paper of the gypsum wallboard above the water line as well as on the front paper below the water line (6.40E^{-05}), the gypsum itself below the water line (8.69E^{-06}), and the paper rear below the water line (7.99E^{-06}) contained low concentrations of this fungus.

المنارة للاستشارات
www.manaraa.com

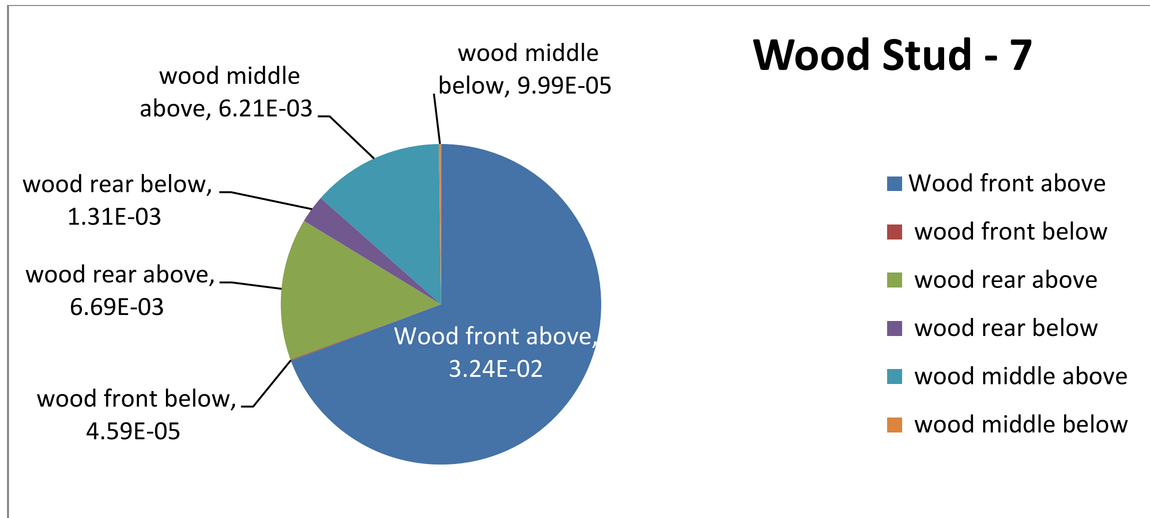

Figure 3.62 The distribution of *Paecilomyces variotii*, based on DNA concentrations, on the different components of the wet wood stud-7.

Wood Stud-7 in Figure 3.62 shows that *Paecilomyces variotii* was present in all samples of the wood stud. *P. variotii* was found present in high concentrations on the front wood stud above the water line ($3.24E^{-02}$). Moderately high concentrations were found on the rear section above the water line ($1.31E^{-03}$), the wood stud middle section above the water line ($6.21E^{-03}$), and wood stud rear section above the water line ($6.69E^{-03}$). The other wood stud components showed moderately low concentrations of *P. variotii*.

المنارة للاستشارات
www.manaraa.com

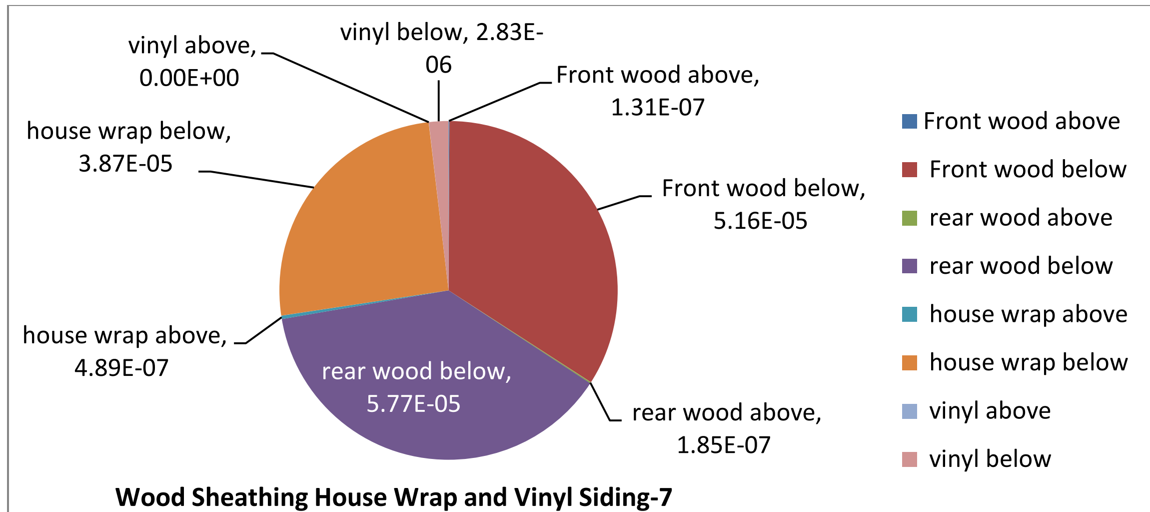

Figure 3.63 The distribution of *Paecilomyces variotii*, based on DNA concentrations, on the different components of wood sheathing, plus house wrap, and vinyl siding-7.

The distribution of *Paecilomyces variotii* on wood sheathing, house wrap, and vinyl siding in Figure 3.63 shows the highest concentrations on the wood sheathing. The rear side of the wood sheathing below the water line supported the highest concentration $5.77E^{-05}$, while the wood sheathing on the front side below the water line supported a concentration of $5.16E^{-05}$. The house wrap below the water line also supported a moderate concentration of $3.87E^{-05}$. The other materials contained very low to no concentrations of *P. variotii*. Statistics were performed for the wet materials on both above and below the water line for *P. variotii* and shown in Table 3.26.

المنارة للاستشارات
www.manaraa.com

Table 3.25 Statistical analysis on *Paecilomyces variotii* both above and below the water line on all wet materials.

State	Waterline	Samples	Mean	Std Dev
wet	above	57	0.002419	0.009004
wet	below	57	9.11E-05	0.000306

Table 3.25 shows that *Paecilomyces variotii* was found in higher concentrations above the water line than below the water line on the wet materials. The dry samples were examined for *Paecilomyces variotii* on the wood stud and gypsum wallboard. The different dry materials examined both above and below the water line are shown in Figure 3.64.

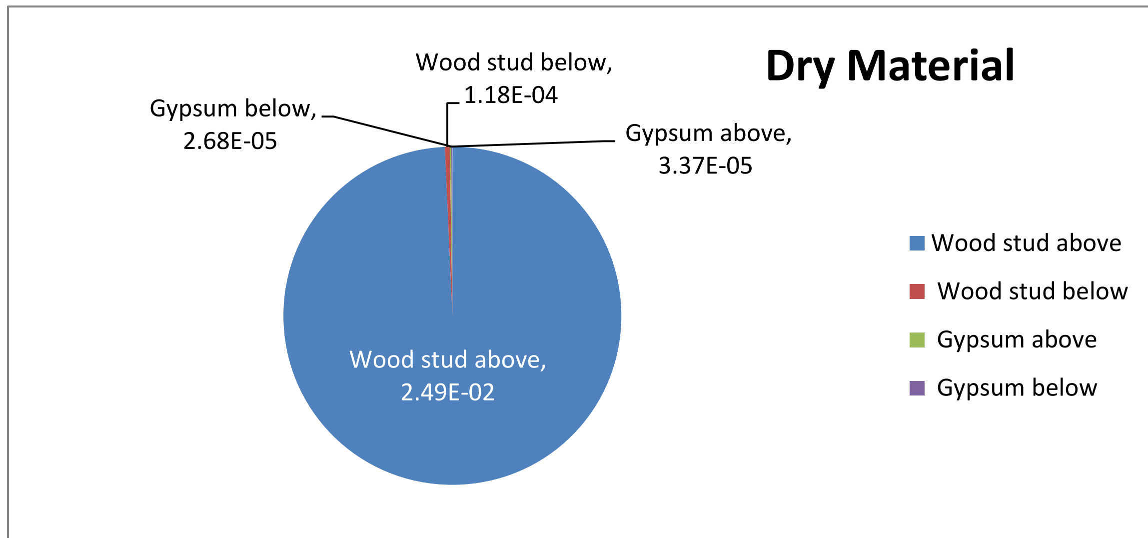

Figure 3.64 The distribution of *Paecilomyces variotii*, based on DNA concentrations, on the different sections of the dry building materials.

Figure 3.64 show that *Paecilomyces variotii* was present on both materials although *P. variotti* was primarily located on the wood stud. The wood stud above the

المنارة للاستشارات
www.manaraa.com

water line had the highest concentration of $2.49E^{-02}$. This is followed by the wood stud below the water line ($1.18E^{-04}$), and the gypsum wallboard below the water line ($2.68E^{-05}$) and above ($3.37E^{-05}$). There were no statistical differences found in *P. variotii* concentrations among the different dry materials (Table 3.26).

Table 3.26 Statistical (ANOVA) anaylsis of the significant difference of dry building materials. (Means with the same letter are not significantly different).

ANOVA Statistical Difference			
Tukey Grouping	**Mean**	**N**	**Material**
A	0.012521	18	Wood stud
A	0.000030	18	Gypsum

The concentrations of *Paecilomyecs variotii* were examined on individual components of the dry samples and are shown in Figure 3.65 and Figure 3.66.

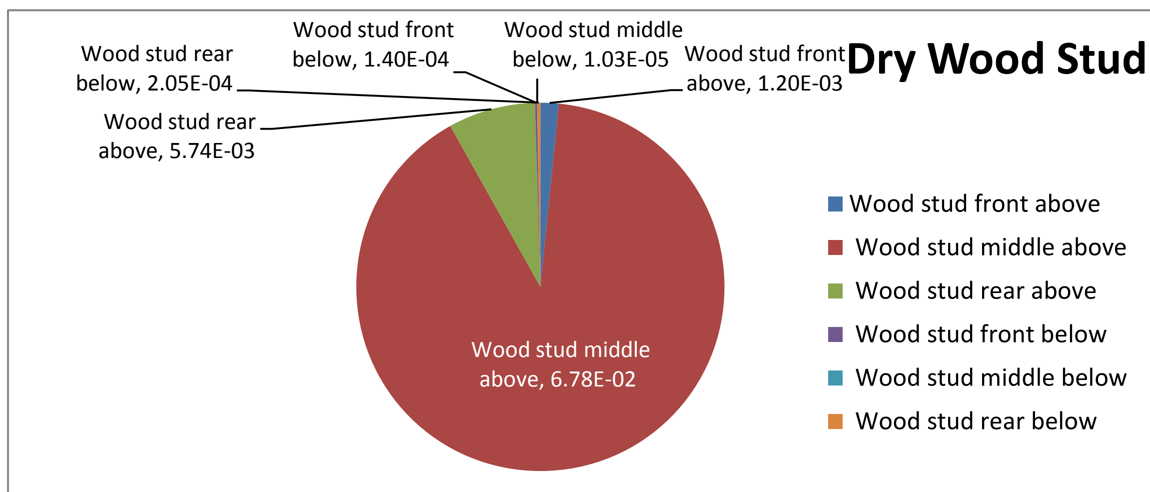

Figure 3.65 The distribution of *Paecilomyces variotii*, based on DNA concentrations, on the different components of the dry wood stud.

المنارة للاستشارات
www.manaraa.com

The dry wood stud (Figure 3.65) supported *Paecilomyces variotii* primarily above the water line. The middle section of the wood stud above the water line contained the highest concentration of $6.78E^{-02}$, while $5.74E^{-03}$ was detected on the rear section of the wood stud above the water line and $1.20E^{-03}$ on the front section of the wood stud above the water line. The remaining wood components contained moderate levels of this fungus included the middle below the water line ($1.03E^{-05}$), the front section below the water line ($1.40E^{-04}$), and the rear section below the water line had a concentration of $2.05E^{-04}$).

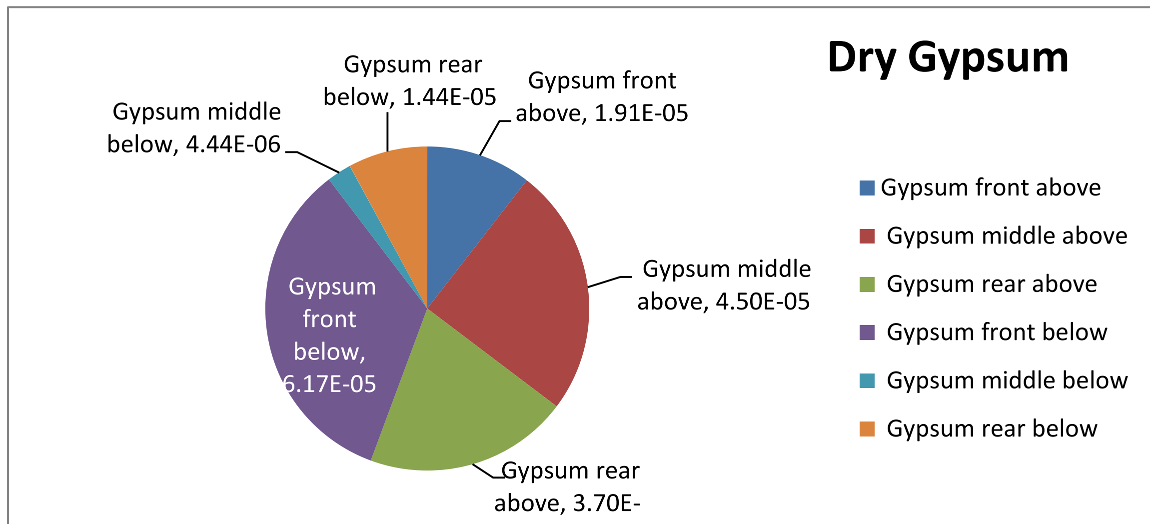

Figure 3.66 The distribution of *Paecilomyces variotii*, based on DNA concentrations, on the different components of the dry gypsum.

In Figure 3.66 *Paecilomyces variotii* was found to be distributed throughout the wallboard. Moderate concentrations were found on the paper front of the gypsum wallboard below the water line ($6.17E^{-05}$), on the gypsum middle above the water line ($4.50E^{-05}$), on the gypsum front above the water line ($1.91E^{-05}$) and the paper rear below

المنارة للاستشارات
www.manaraa.com

the water line ($1.44E^{-05}$). It is surprising to see gypsum itself support the presence of this fungus.

Paecilomyces variotii was distributed in moderate concentrations on all of the wall materials analyzed. The wet wood stud contained significantly greater concentrations of this fungus and the highest individual concentration $3.24E^{-02}$ above the water line for all wet materials. *P. variotii* was 25x more likely to be found on all wet wall materials above the water line compared to below the water line. The dry wood stud supported the highest concentration of *P. variotii* for all wall materials. The above the water line average was $7.47E^{-02}$.

Stachybotrys chartarum

Stachybotrys chartarum is a deuteromycete fungus in the family Dematiaceae, and commonly found on very wet gypsum wallboards and wallpaper after water damage (Flannigan et al. 2011). *Stachybotrys chartarum* colonies on Potato Dextrose Agar (PDA) at 25°C can reach up to 28mm in 7 days, appearing blackish-green with a white rim and powdery due to the conidial masses. Colonies on Synthetic Nutrient Deficient Agar (SNA) have a diameter of 30mm after 7 days at 25°C in the dark, appearing thread-like with blackish-green center of conidia. *Stachybotrys chartarum* contains two chemotypes (S and A) which have the same morphology, but different metabolites. *Stachybotrys* (S) produces satratoxins and roridins, while *Stacybotrys chartarum* (A) produces atranones, dolabellanes, and trichodermin. Exposure to *Stachybotrys* on natural substrates or in culture can cause rashes and mucosal irritation or bleeding (Samson et al. 2010). The DNA concentrations of *Stachybotrys chartarum* were determined using real-time PCR of

المنارة للاستشارات
www.manaraa.com

each wall construction material and converted to the amount of fungal DNA in nannograms per weight of the material sample (Table 3.27).

Table 3.27 Real-time PCR results of *Stachybotrys chartarum* DNA in nannograms per weight of material

Sample Code	Material Weight (mg)	Extraction volume	Amount µL used	Real Time (ng)	Average Reps	(ng)DNA/(mg) Material	Average DNA/Material
B1AP1	100	80	3 µL	8.42E-02		2.25E-02	
B1AP2	100	80	3 µL	8.86E-04	2.86E-02	2.36E-04	7.64E-03
B1AP3	100	80	3 µL	8.63E-04		2.30E-04	
B1BP1	140	80	3 µL	0.00E+00		0.00E+00	
B1BP2	140	80	3 µL	0.00E+00	1.95E-05	0.00E+00	3.72E-06
B1BP3	140	80	3 µL	5.86E-05		1.12E-05	
B1AN1	50	80	3 µL	2.73E-04		1.46E-04	
B1AN2	50	80	3 µL	1.80E-05	9.70E-05	9.60E-06	5.17E-05
B1AN3	50	80	3 µL	0.00E+00		0.00E+00	
B1BN1	100	80	3 µL	0.00E+00		0.00E+00	
B1BN2	100	80	3 µL	9.33E-07	4.58E-06	2.49E-07	1.22E-06
B1BN3	100	80	3 µL	1.28E-05		3.41E-06	
B1AF1	50	80	3 µL	1.07E-06		5.71E-07	
B1AF2	50	80	3 µL	0.00E+00	3.57E-07	0.00E+00	1.90E-07
B1AF3	50	80	3 µL	0.00E+00		0.00E+00	
B1BF1	100	80	3 µL	0.00E+00		0.00E+00	
B1BF2	100	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
B1BF3	100	80	3 µL	0.00E+00		0.00E+00	
G3AS1	200	80	3 µL	3.55E-06		4.73E-07	
G3AS2	200	80	3 µL	0.00E+00	1.18E-06	0.00E+00	1.58E-07
G3AS3	200	80	3 µL	0.00E+00		0.00E+00	
G3BS1	250	80	3 µL	0.00E+00		0.00E+00	
G3BS2	250	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G3BS3	250	80	3 µL	0.00E+00		0.00E+00	
G3AR1	250	80	3 µL	0.00E+00		0.00E+00	
G3AR2	250	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G3AR3	250	80	3 µL	0.00E+00		0.00E+00	
G3BR1	300	80	3 µL	0.00E+00		0.00E+00	

www.manaraa.com

Table 3.27 (continued)

G3BR2	300	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G3BR3	300	80	3 µL	0.00E+00		0.00E+00	
G3AM1	500	80	3 µL	0.00E+00		0.00E+00	
G3AM2	500	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G3AM3	500	80	3 µL	0.00E+00		0.00E+00	
G3BM1	500	80	3 µL	0.00E+00		0.00E+00	
G3BM2	500	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G3BM3	500	80	3 µL	0.00E+00		0.00E+00	
G4AS1	200	80	3 µL	3.64E-07		4.85E-08	
G4AS2	230	80	3 µL	0.00E+00	1.21E-07	0.00E+00	1.62E-08
G4AS3	200	80	3 µL	0.00E+00		0.00E+00	
G4BS1	210	80	3 µL	0.00E+00		0.00E+00	
G4BS2	200	80	3 µL	4.86E-06	1.62E-06	6.48E-07	2.16E-07
G4BS3	200	80	3 µL	0.00E+00		0.00E+00	
G4AR1	100	80	3 µL	1.11E-05		2.96E-06	
G4AR2	100	80	3 µL	0.00E+00	3.70E-06	0.00E+00	9.87E-07
G4AR3	100	80	3 µL	0.00E+00		0.00E+00	
G4BR1	200	80	3 µL	0.00E+00		0.00E+00	
G4BR2	200	80	3 µL	4.16E-06	1.39E-06	5.55E-07	1.85E-07
G4BR3	200	80	3 µL	0.00E+00		0.00E+00	
G4AM1	300	80	3 µL	0.00E+00		0.00E+00	
G4AM2	300	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G4AM3	320	80	3 µL	0.00E+00		0.00E+00	
G4BM1	500	80	3 µL	0.00E+00		0.00E+00	
G4BM2	520	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G4BM3	500	80	3 µL	0.00E+00		0.00E+00	
G2TS1	150	80	3 µL	2.19E-07		3.89E-08	
G2TS2	160	80	3 µL	0.00E+00	1.26E-05	0.00E+00	2.24E-06
G2TS3	150	80	3 µL	3.76E-05		6.68E-06	
G2BS1	160	80	3 µL	0.00E+00		0.00E+00	
G2BS2	150	80	3 µL	6.74E-05	2.25E-05	1.20E-05	3.99E-06
G2BS3	150	80	3 µL	0.00E+00		0.00E+00	
G2TR1	100	80	3 µL	1.94E-02		5.17E-03	
G2TR2	100	80	3 µL	0.00E+00	6.47E-03	0.00E+00	1.72E-03
G2TR3	100	80	3 µL	1.94E-06		5.17E-07	
G2BR1	100	80	3 µL	2.43E-05		6.48E-06	
G2BR2	80	80	3 µL	9.03E-06	1.11E-05	3.01E-06	3.16E-06
G2BR3	80	80	3 µL	0.00E+00		0.00E+00	

المنارة للاستشارات
www.manaraa.com

Table 3.27 (continued)

G2TM1	3150	80	3 µL	3.23E-05		2.73E-07	
G2TM2	100	80	3 µL	3.62E-05	2.28E-05	9.65E-06	3.31E-06
G2TM3	200	80	3 µL	0.00E+00		0.00E+00	
G2BM1	170	80	3 µL	0.00E+00		0.00E+00	
G2BM2	280	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G2BM3	200	80	3 µL	N/A		N/A	
W7AS1	100	80	3 µL	0.00E+00		0.00E+00	
W7AS2	100	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
W7AS3	140	80	3 µL	0.00E+00		0.00E+00	
W7BS1	50	80	3 µL	1.82E-05		9.71E-06	
W7BS2	50	80	3 µL	0.00E+00	6.07E-06	0.00E+00	3.24E-06
W7BS3	40	80	3 µL	0.00E+00		0.00E+00	
W7AR1	40	80	3 µL	0.00E+00		0.00E+00	
W7AR2	80	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
W7AR3	80	80	3 µL	0.00E+00		0.00E+00	
W7BR1	50	80	3 µL	0.00E+00		0.00E+00	
W7BR2	60	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
W7BR3	50	80	3 µL	0.00E+00		0.00E+00	
I7A1	40	80	3 µL	0.00E+00		0.00E+00	
I7A2	40	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
I7A3	40	80	3 µL	0.00E+00		0.00E+00	
I7B1	60	80	3 µL	0.00E+00		0.00E+00	
I7B2	50	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
I7B3	50	80	3 µL	0.00E+00		0.00E+00	
V7A1	400	80	3 µL	0.00E+00		0.00E+00	
V7A2	400	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
V7A3	400	80	3 µL	0.00E+00		0.00E+00	
V7B1	400	80	3 µL	0.00E+00		0.00E+00	
V7B2	400	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
V7B3	300	80	3 µL	0.00E+00		0.00E+00	
W4AS1	80	80	3 µL	0.00E+00		0.00E+00	
W4AS2	90	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
W4AS3	760	80	3 µL	0.00E+00		0.00E+00	
W4BS1	900	80	3 µL	0.00E+00		0.00E+00	
W4BS2	76	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
W4BS3	130	80	3 µL	0.00E+00		0.00E+00	
W4BR1	150	80	3 µL	0.00E+00		0.00E+00	
W4AR2	90	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00

www.manaraa.com

Table 3.27 (continued)

W4AR3	70	80	3 µL	0.00E+00		0.00E+00	
W4BR1	40	80	3 µL	0.00E+00		0.00E+00	
W4BR2	65	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
W4BR3	50	80	3 µL	0.00E+00		0.00E+00	
W4AM1	60	80	3 µL	0.00E+00		0.00E+00	
W4AM2	50	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
W4AM3	40	80	3 µL	0.00E+00		0.00E+00	
W4BM1	53	80	3 µL	0.00E+00		0.00E+00	
W4BM2	50	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
W4BM3	66	80	3 µL	0.00E+00		0.00E+00	
G1AS1	120	80	3 µL	0.00E+00		0.00E+00	
G1AS2	170	80	3 µL	0.00E+00	2.28E-05	0.00E+00	3.04E-06
G1AS3	200	80	3 µL	6.83E-05		9.11E-06	
G1BS1	200	80	3 µL	0.00E+00		0.00E+00	
G1BS2	300	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G1BS3	500	80	3 µL	0.00E+00		0.00E+00	
G1AR1	62	80	3 µL	0.00E+00		0.00E+00	
G1AR2	75	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G1AR3	100	80	3 µL	0.00E+00		0.00E+00	
G1BR1	97	80	3 µL	0.00E+00		0.00E+00	
G1BR2	100	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G1BR3	150	80	3 µL	0.00E+00		0.00E+00	
G1AM1	500	80	3 µL	0.00E+00		0.00E+00	
G1AM2	430	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G1AM3	427	80	3 µL	0.00E+00		0.00E+00	
G1BM1	450	80	3 µL	0.00E+00		0.00E+00	
G1BM2	360	80	3 µL	N/A	0.00E+00	N/A	0.00E+00
G1BM3	540	80	3 µL	N/A		N/A	
S1AF1	50	80	3 µL	0.00E+00		0.00E+00	
S1AF2	50	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
S1AF3	50	80	3 µL	0.00E+00		0.00E+00	
S1AM1	50	80	3 µL	1.32E-02		7.04E-03	
S1AM2	50	80	3 µL	4.01E-05	6.62E-03	2.14E-05	3.53E-03
S1AM3	60	80	3 µL	N/A		N/A	
S1AR1	50	80	3 µL	0.00E+00		0.00E+00	
S1AR2	50	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
S1AR3	50	80	3 µL	0.00E+00		0.00E+00	
S2BF1	50	80	3 µL	1.46E-05		7.79E-06	

المنارة للاستشارات
www.manaraa.com

Table 3.27 (continued)

S2BF2	60	80	3 µL	1.71E-05	1.06E-05	7.60E-06	5.13E-06
S2BF3	50	80	3 µL	0.00E+00		0.00E+00	
S2BM1	60	80	3 µL	0.00E+00		0.00E+00	
S2BM2	50	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
S2BM3	60	80	3 µL	0.00E+00		0.00E+00	
S2BR1	50	80	3 µL	0.00E+00		0.00E+00	
S2BR2	50	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
S2BR3	50	80	3 µL	0.00E+00		0.00E+00	
G3AF1	200	80	3 µL	0.00E+00		0.00E+00	
G3AF2	100	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G3AF3	200	80	3 µL	0.00E+00		0.00E+00	
G3AM1	200	80	3 µL	1.91E-05		2.55E-06	
G3AM2	200	80	3 µL	0.00E+00	6.37E-06	0.00E+00	8.49E-07
G3AM3	200	80	3 µL	0.00E+00		0.00E+00	
G3AR1	100	80	3 µL	0.00E+00		0.00E+00	
G3AR2	100	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G3AR3	100	80	3 µL	0.00E+00		0.00E+00	
G4BF1	100	80	3 µL	9.45E-04		2.52E-04	
G4BF2	200	80	3 µL	5.12E-01	1.72E-01	6.83E-02	2.29E-02
G4BF3	200	80	3 µL	1.96E-03		2.61E-04	
G4BM1	300	80	3 µL	0.00E+00		0.00E+00	
G4BM2	200	80	3 µL	0.00E+00	0.00E+00	0.00E+00	0.00E+00
G4BM3	200	80	3 µL	0.00E+00		0.00E+00	
G4BR1	100	80	3 µL	9.68E-05		2.58E-05	
G4BR2	100	80	3 µL	1.43E-04	9.04E-05	3.81E-05	2.41E-05
G4BR3	100	80	3 µL	3.14E-05		8.37E-06	

The wet building materials were analyzed for *Stachybotrys chartarum* on each type of material and this data are shown in Figure 3.67.

www.manaraa.com

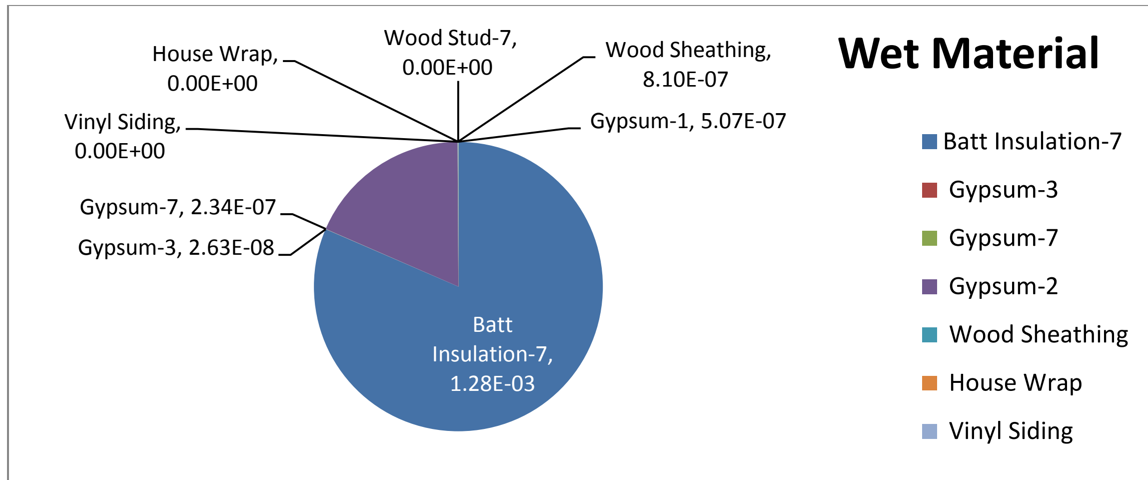

Figure 3.67 The distribution of *Stachybotrys chartarum*, based on averaged DNA concentrations, on the different wet building materials. Samples with a 7 came from the same wall unit.

The wet material averages for *Stachybotrys chartarum* (Figure 3.67) shows that the mold was detected in all materials except for the wood stud-7, house wrap, and vinyl siding. The batt insulation had the highest concentration of 1.28E^{-03} followed by gypsum-2 (2.89E^{-03}). The other gypsum boards contained low levels of *S. chartarum*, gypsum-3 (2.63E^{-08}), and gypsum-7 (2.34E^{-07}). There were no statistical differences found in *S. chartarum* concentrations among the different wet materials (Table 3.28).

المنارة للاستشارات
www.manaraa.com

Table 3.28 Statistical (ANOVA) anaylsis of the significant difference of wet building materials. (Means with the same letter are not significantly different).

ANOVA Statistical Difference			
Tukey Grouping	**Mean**	**N**	**Material**
A	0.0012828	8	Batt Insulation
A	0.0003066	7	Gypsum-2
A	0.0000008	2	Wood sheathing
A	0.0000006	6	Gypsum-1
A	0.0000002	8	Gypsum-7
A	0.0000000	8	Gypsum-3
A	0.0000000	6	House wrap
A	0.0000000	6	Vinyl siding
A	0.0000000	8	Wood stud

The presence of *Stachybotrys chartarum* was examined on individual components of each material and this data are shown in Figures 3.68-3.73.

للاستشارات المنارة
www.manaraa.com

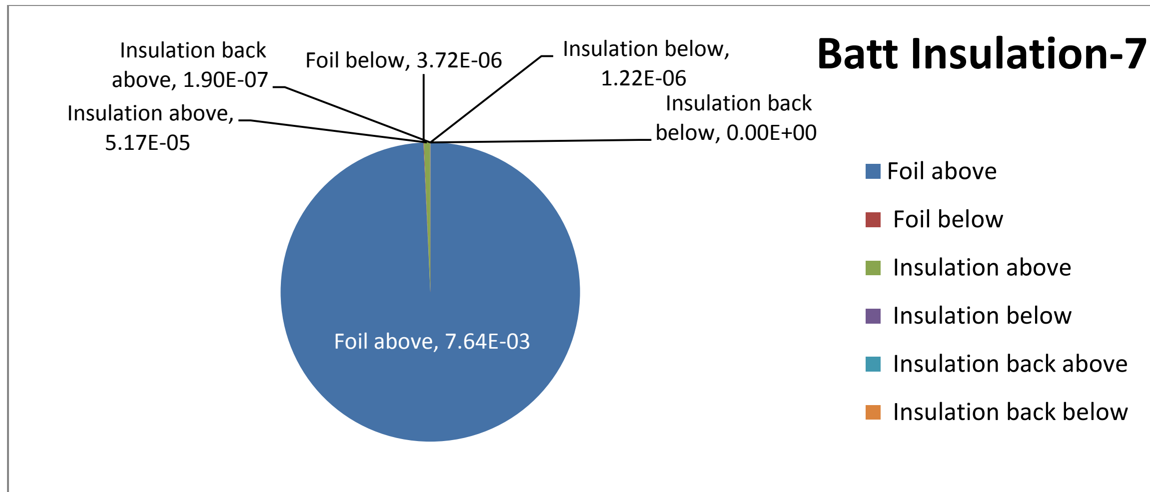

Figure 3.68 The distribution of *Stachybotrys chartarum*, based on DNA concentrations, on the different components of the wet batt insulation-7.

The distribution of *Stachybotrys chartarum* in the batt insulation (Figure 3.68) ranged from the highest concentration of 7.64E^{-03} above the water line on the foil (blue) followed by the insulation above (5.17E^{-05}) and the foil below the water line (3.72E^{-06}). The other components had low concentrations of *Stachybotrys chartarum*.

المنارة للاستشارات
www.manaraa.com

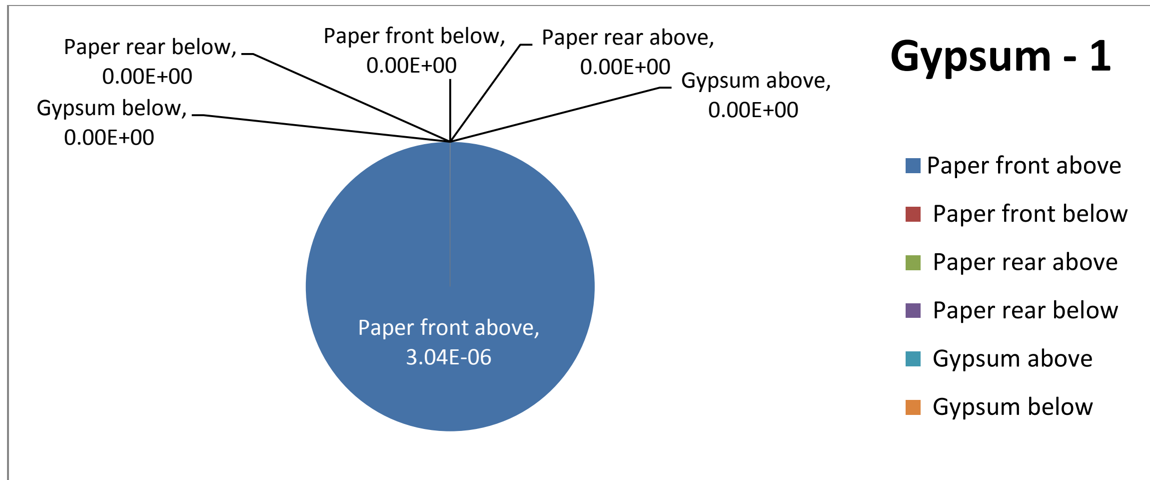

Figure 3.69 The distribution of *Stachybotrys chartarum*, based on DNA concentrations, on the different components of the dry gypsum-1.

Figure 3.69 shows a presence of *Stachybotrys chartarum* in low to no concentrations in all locations of the gypsum-1 wallboard. *Stachybotrys chartarum* was found on the front paper above the water line ($3.04E^{-06}$), while the other components showed no *S. chartarum*.

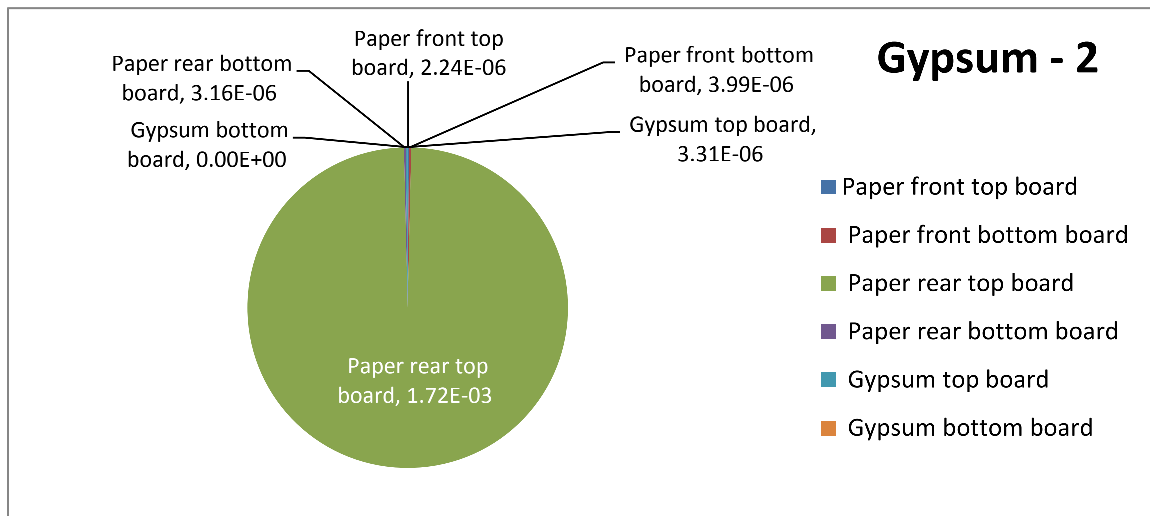

Figure 3.70 The distribution of *Stachybotrys chartarum*, based on DNA concentrations, on the different sections of the wet gypsum-2.

المنارة للاستشارات
www.manaraa.com

Figure 3.70 shows the presence of *Stachybotrys chartarum* in the highest concentration on the rear paper on the top of the board ($1.72E^{-03}$) of gypsum-2. The other components showed low concentrations including the gypsum itself on the top of the board ($3.31E^{-06}$), the paper front at the bottom of the board ($3.99E^{-06}$) and top of the board ($2.24E^{-06}$), and the paper rear at the bottom of the board ($3.16E^{-06}$).

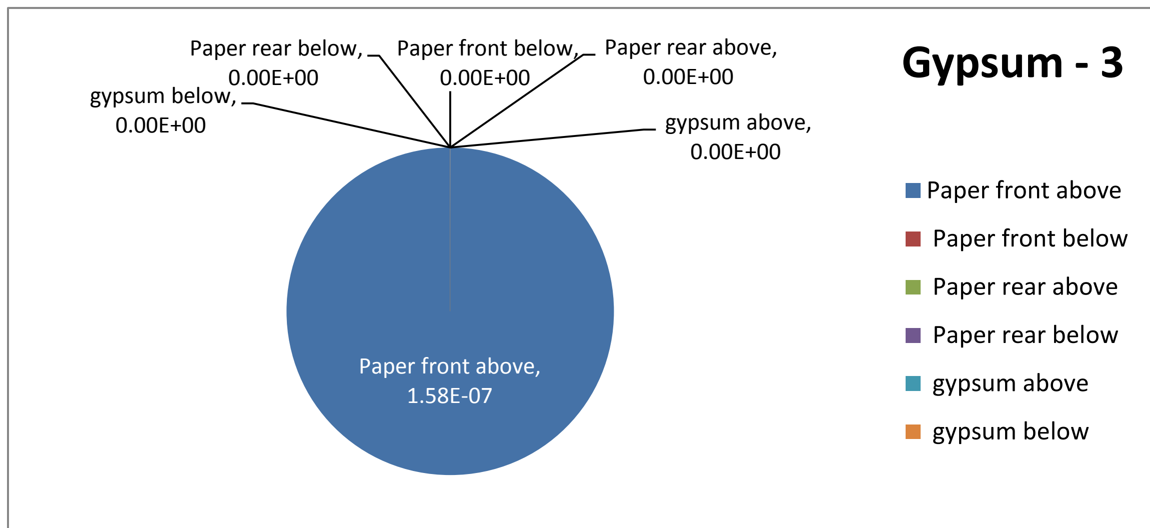

Figure 3.71 The distribution of *Stachybotrys chartarum*, based on DNA concentrations, on the different components of the wet gypsum-3.

Gypsum -3 (Figure 3.71) shows very low concentrations of *Stachybotrys chartarum* on the front paper of the wallboard above the water line ($1.58E^{-07}$). However, the other gypsum components supported no presence of *S. chartarum*.

المنارة للاستشارات
www.manaraa.com

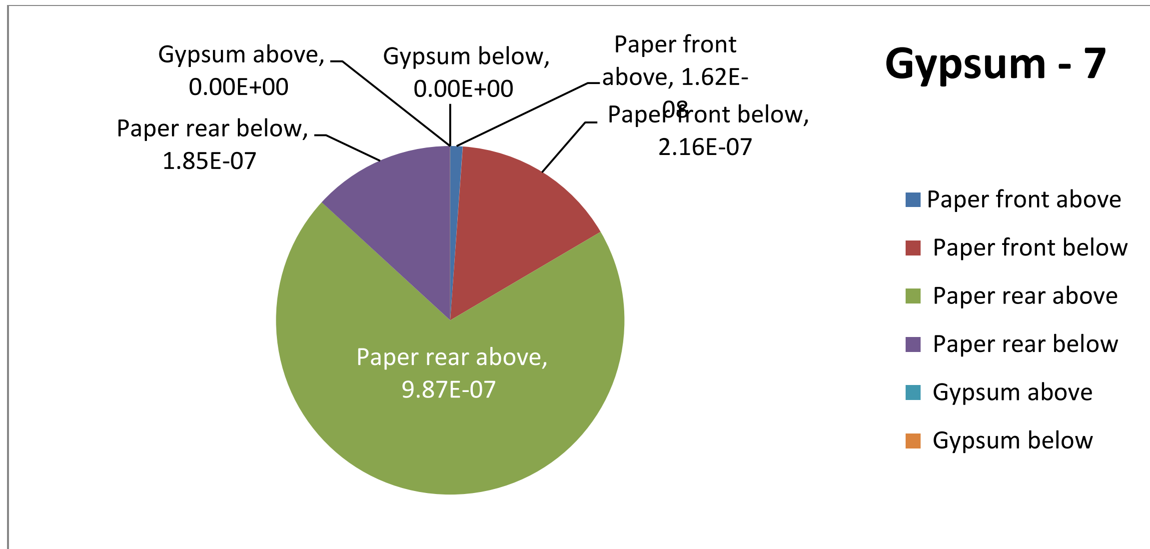

Figure 3.72 The distribution of *Stachybotrys chartarum*, based on DNA concentrations, on the different components of the gypsum-7.

Gypsum -7 (Figure 3.72) supported *Stachybotrys chartarum* in low concentrations on the rear paper of the gypsum wallboard above the water line ($9.87E^{-07}$), the paper front below the water line ($2.16E^{-07}$), the rear paper below the water line ($1.85E^{-07}$), and the paper front above the water line ($1.62E^{-08}$).

www.manaraa.com

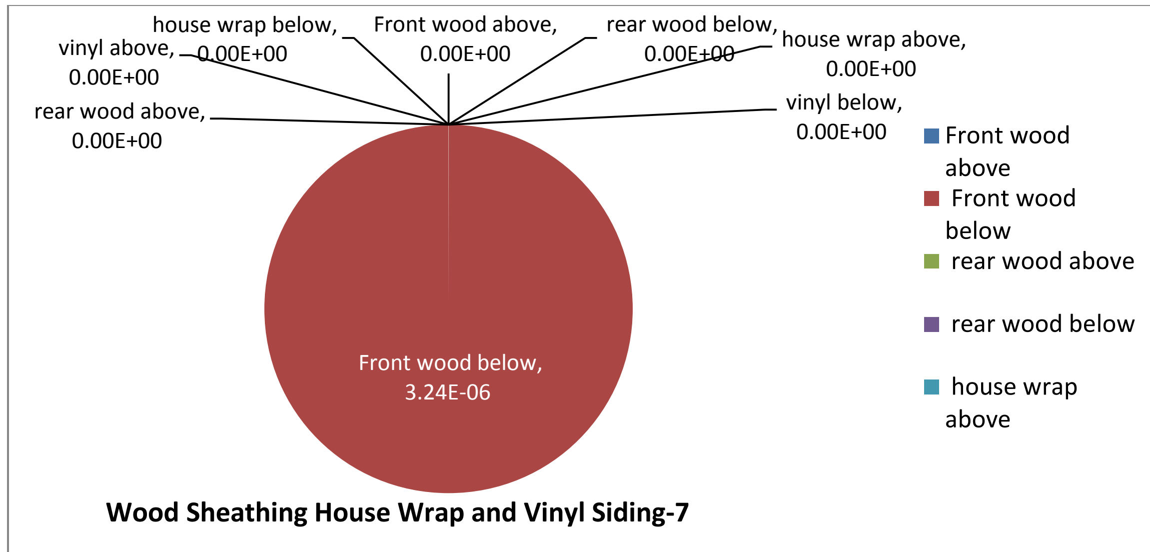

Figure 3.73 The distribution of *Stachybotrys chartarum*, based on DNA concentrations, on the different components of the wet wood sheathing, plus house wrap, and vinyl siding-7.

Stachybotrys chartarum was detected only on the front component of the wood sheathing below the water line (Figure 3.73). The wood sheathing had a moderately concentration of ($3.24E^{-06}$). Statistics were performed for the wet materials on both above and below the water line for *S. chartarum* and is presented in Table 3.29.

Table 3.29 Statistical analysis on *Stachybotrys chartarum* both above and below the water line on the wet materials.

State	Waterline	Samples	Mean	Std Dev
wet	above	57	0.000405	0.002973
wet	below	57	4.68E-07	2.01E-06

Table 3.29 shows that *Stachybotrys chartarum* was present in higher concentrations above the water line than below the water line. The dry samples were also

المنارة للاستشارات
www.manaraa.com

examined for *Stachybotrys chartarum* on the wood stud and gypsum wallboard. The different dry materials examined both above and below the water line are shown in Figure 3.74.

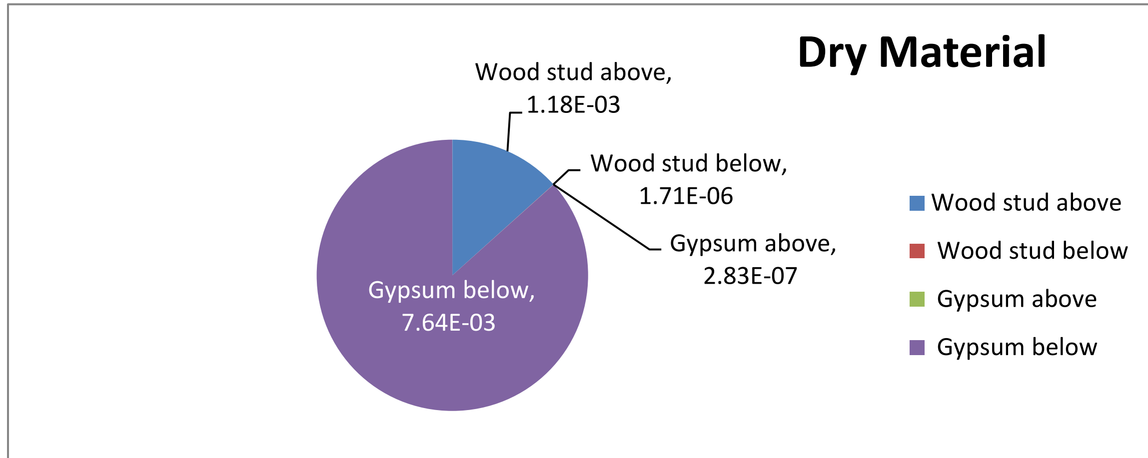

Figure 3.74 The distribution of *Stachybotrys chartarum*, based on DNA concentrations, on the different dry building materials.

In Figure 3.74 , *Stachybotrys chartarum* was found in the highest concentrations below the water line in the middle section of gypsum ($2.29E^{-02}$) followed by the wood stud on the middle section above the water line ($3.53E^{-03}$). The other wall construction materials, the middle section of the gypsum above the water line ($8.49E^{-07}$), wood stud on the front section below the water line ($5.13E^{-06}$), and gypsum on the rear section below the water line ($2.41E^{-05}$) all had moderate concentrations of *S. chartarum*. There were no statistical differences found in *Stachybotrys chartarum* concentrations among the different dry materials (Table 3.30).

المنارة للاستشارات
www.manaraa.com

Table 3.30 Statistical (ANOVA) anaylsis of the significant difference of dry building materials. (Means with the same letter are not significantly different).

ANOVA Statistical Difference			
Tukey Grouping	**Mean**	**N**	**Material**
A	0.003825	8	Gypsum
A	0.000416	7	Wood stud

Figures 3.75- 3.76 show the concentrations of *Stachybotrys chartarum* on dry wood stud and gypsum.

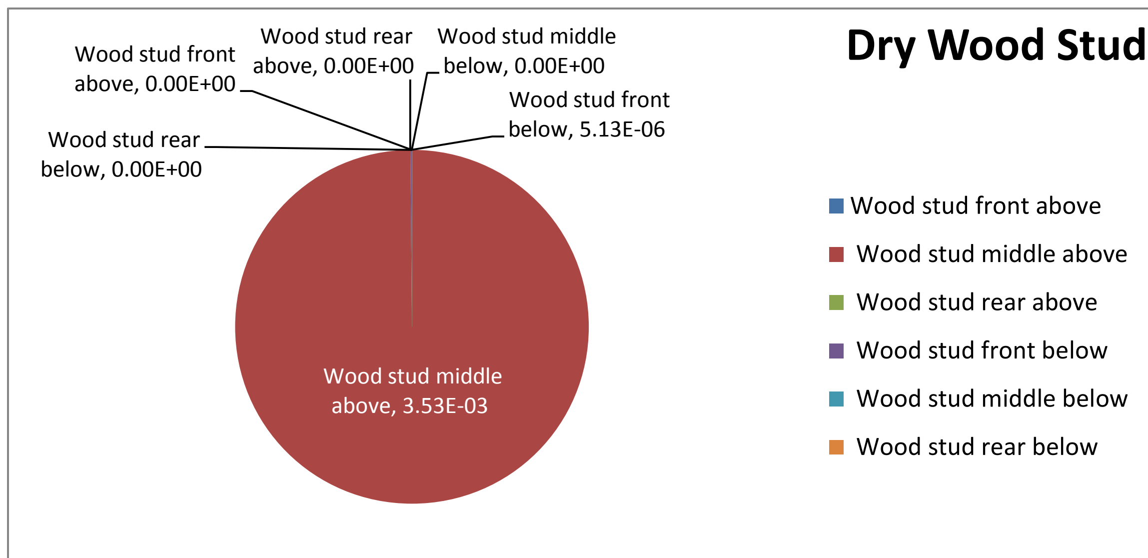

Figure 3.75 The distribution of *Stachybotrys chartarum*, based on DNA concentrations, on the different components of the dry wood stud.

The dry wood stud in Figure 3.75 shows that *Stachybotrys chartarum* was primarily present on the middle section of the wood stud above the water line at a

المنارة للاستشارات
www.manaraa.com

concentration of 3.53E^{-03}. The other components of the dry wood stud had little to no concentration of *S. chartarum*.

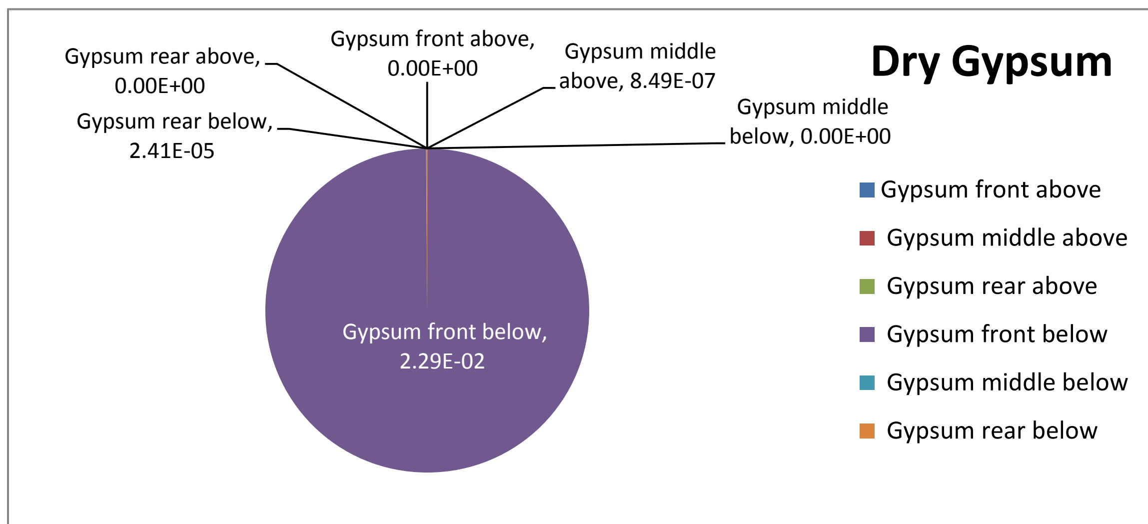

Figure 3.76 The distribution of *Stachybotrys chartarum*, based on DNA concentrations, on the different components of the dry gypsum.

The dry gypsum wallboard in Figure 3.76 shows that the majority of *Stachybotrys chartarum* was present on the front paper of the wallboard below the water line at a high concentration of 2.92E^{-02}. All other gypsum components showed little to no concentration of *S. chartarum*.

Stachybotrys chartarum was found in high or moderately high concentrations on only a few of the wall materials. On the wet materials, the highest concentration (E^{-03}) was on the foil above the water line and the paper at the top of gypsum-2. *S. chartarum* was 800x more likely to be encountered on the wet material above the water line versus below. The highest concentration of all material samples was found on the dry gypsum paper at the front of the board (E^{-02}) followed by the dry wood stud (E^{-03}). It is interesting

المنارة للاستشارات
www.manaraa.com

that *S. chartarum* with the exception of gypsum-2, it was only present in low concentrations on the wet gypsum wallboards, yet in the highest concentration on the dry gypsum. Although sporadic in its occurance, the presence of *Stachybotrys chartarum* both on the wet and dry materials is a concern.

Composition of Materials

Quantization of Mold on Wet Materials

Each mold species was quantitated on each building material to determine which species was the most prevalent based on its concentration. The following Figures 3.77-3.87 shows the distribution of each organism on each wall material.

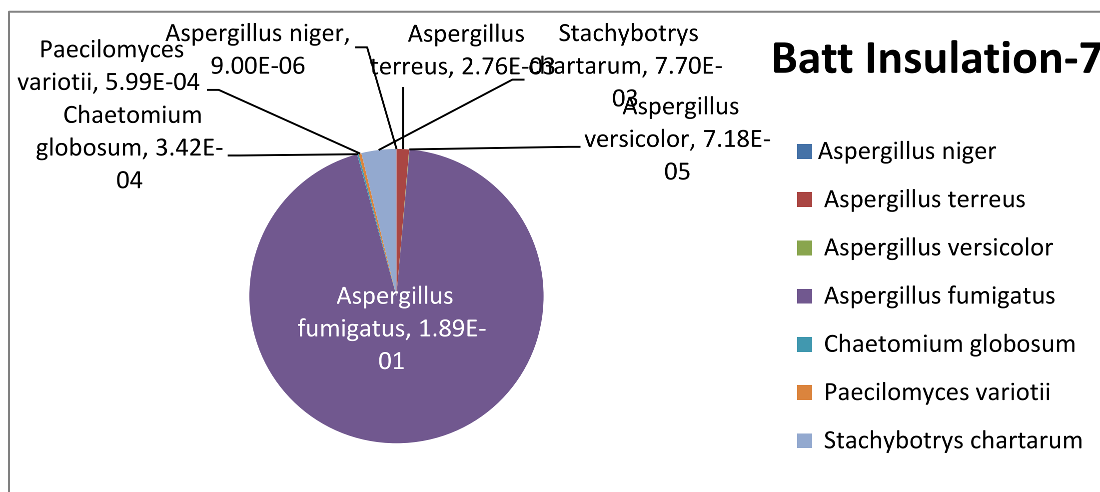

Figure 3.77 The distribution of the selected mold species, based on total DNA concentrations, on the batt insulation-7.

The batt insulation shown in Figure 3.77 supported the highest average concentration for four of the seven fungi measured. *Aspergillus fumigatus* was present in a very high concentration of $1.89E^{-01}$. In fact, this was the highest concentration for any

المنارة للاستشارات
www.manaraa.com

mold on any of the wall materials. The other mold species that were most abundant on the batt were *Stachybotrys chartarum* (7.70E^{-03}), *Aspergillus terreus* (2.76E^{-03}), and *Aspergillus niger* (9.00E^{-06}). The other three fungi were found in higher concentrations on other wall materials compared to their average on the batt. Of the seven fungi, five were found almost exclusively above the water line on the batt insulation. The exceptions were *Aspergillus fumigatus* which was found primarily below the water line on the batt, and *Aspergillus versicolor* which did not show a clear preference. It is possible the moisture content of the batt below the water line remained too high and inhibited the growth of most molds. *A. fumigatus* is the exception as it thrives in higher moisture environments. Overall the batt insulation supported the highest concentration of total fungi for any of the wet materials (2.00E^{-01}) and was essentially tied with the dry wood stud.

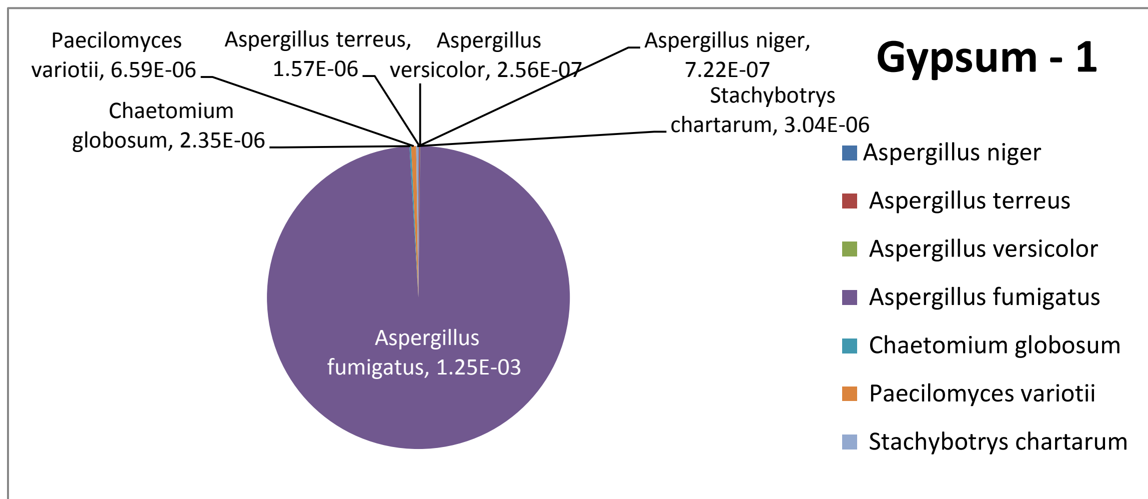

Figure 3.78 The distribution of the selected mold species, based on total DNA concentrations, on the gypsum-1.

www.manaraa.com

As seen in Figure 3.79 the gypsum-1 sample supported the growth of all mold species. *Aspergillus fumigatus* was present in the highest concentration of $1.25E^{-03}$ and contributed 99% of the total fungal DNA detected on this material. All other species were present on gypsum-1 but in very low concentrations. This material supported the least total fungal growth ($1.26E^{-03}$) than any of the other gypsum materials and of the eleven wet and dry materials analyzed, gypsum-1 ranked 9th (with the material supporting the most fungal DNA ranked as 1st).

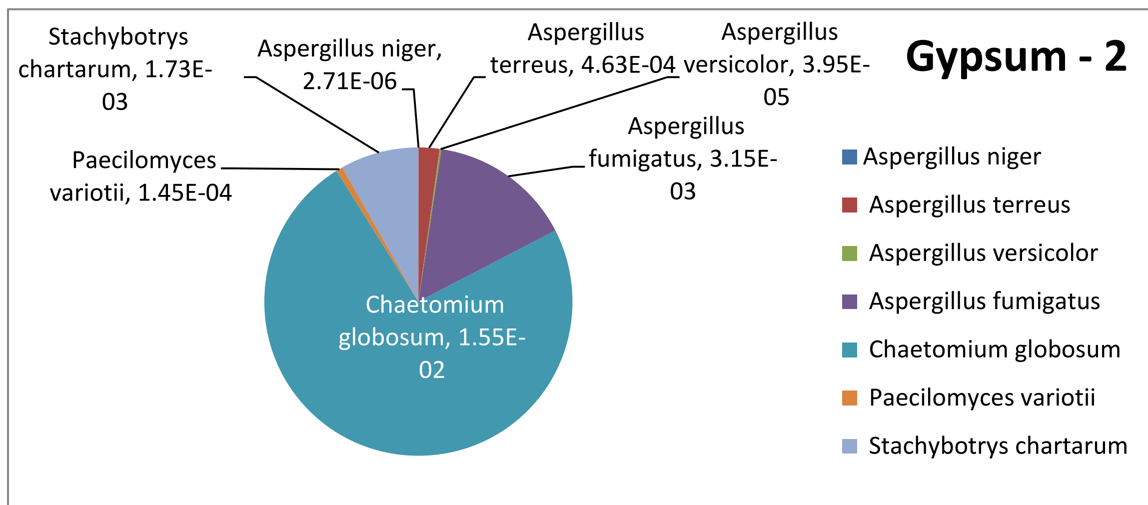

Figure 3.79 The distribution of the selected mold species, based on total DNA concentrations, on the gypsum-2 material.

Gypsum -2 in (Figure 3.79) supported the highest concentration of fungal DNA compared to the other wet gypsum materials. *Chaetomium globosum* was present in the highest concentration of $1.55E^{-02}$ and this comprised 75% of the total fungal DNA on the gypsum-2 sample. Also present in notable quantities were *Aspergillus fumigatus* which

المنارة للاستشارات
www.manaraa.com

made up 15% of the total, *Stachybotrys chartarum* (8%), and *Aspergillus terreus* (2%). The other three mold species contributed less than 1% to the total fungal DNA on the gypsum-2 sample. This is the only gypsum material in which *Chaetomium* was the dominant species. In total fungal DNA, the gypsum-2 material tied for 4th ($2.10E^{-02}$) with the wood sheathing and dry gypsum wallboard.

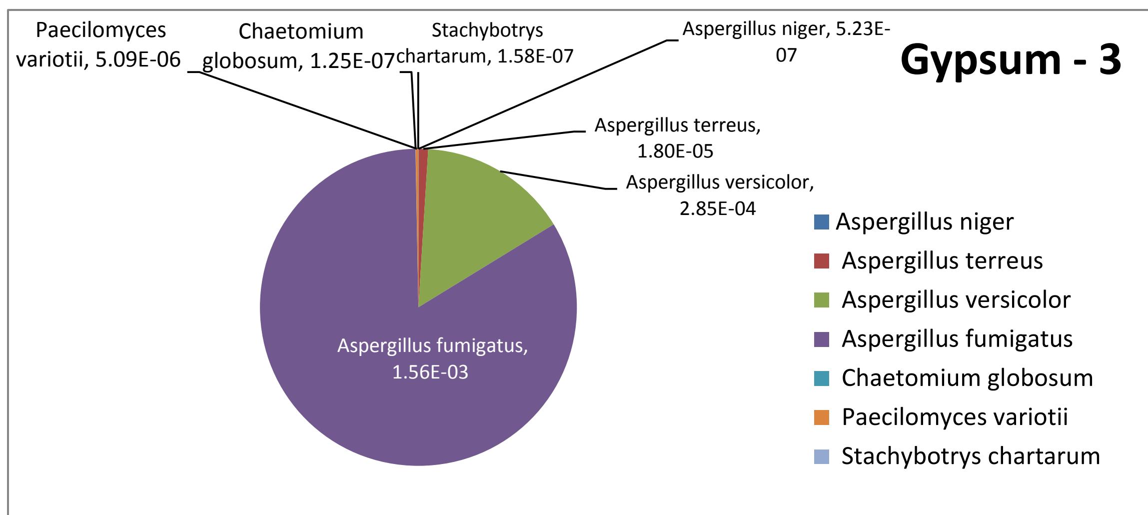

Figure 3.80 The distribution of the selected mold species, based on total DNA concentrations, on the gypsum-3.

In Figure 3.80 the gypsum-3 was dominated by the *Aspergillus* species. *Aspergillus fumigatus* was present in the highest concentration and this species accounted for 83% of the total DNA. This was followd by *Aspergillus versicolor* which accounted for another 15%, then *Aspergillus terreus* and *A. niger* comprised another 1.7%. All total the four *Aspergillus* species made up 99.7% of the total fungal DNA found on gypsum-3. This wall material ranked eight of eleven for total fungal DNA ($1.87E^{-03}$).

المنارة للاستشارات
www.manaraa.com

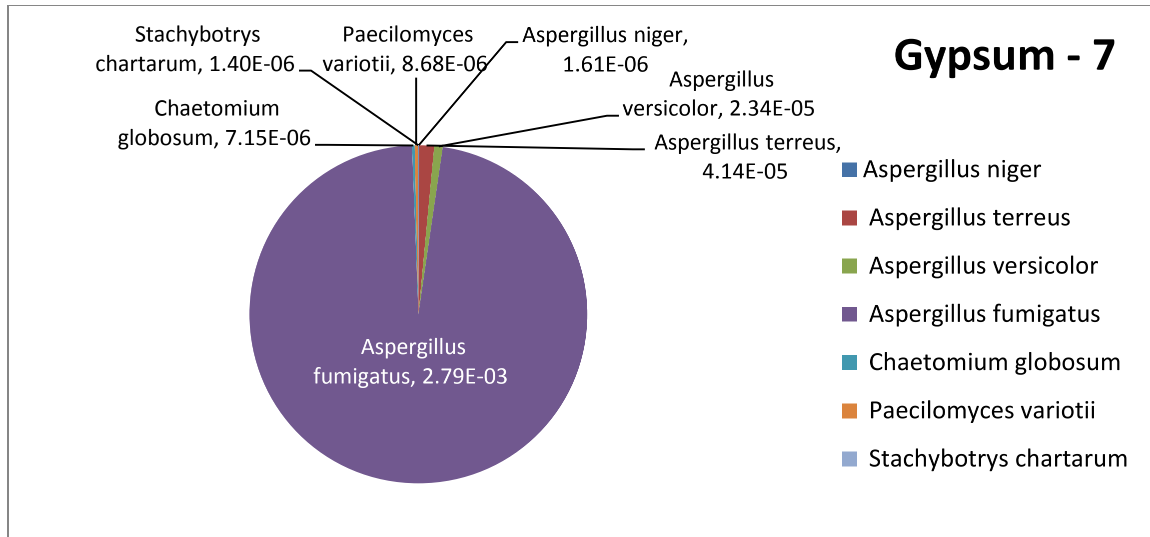

Figure 3.81 The distribution of the selected mold species, based on total DNA concentrations, on the gypsum-7 material.

Aspergillus fumigatus was the dominant fungus on the gypsum-7 samples (Figure 3.81) making up 97% of the total fungal DNA on this material. The only other notable contributions were from *A. terreus* (1.4%) and *A. versicolor* (0.8%). Overall gypsum-7 ranked 7th of the eleven in terms of the total fungal DNA found on this material.

المنارة للاستشارات
www.manaraa.com

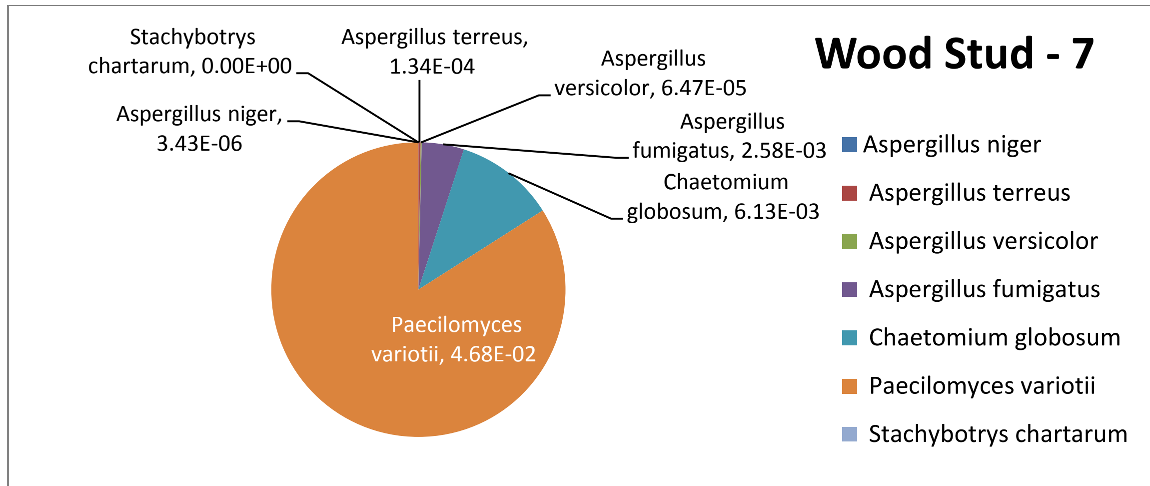

Figure 3.82 The distribution of the selected mold species, based on total DNA concentrations, on wood stud-7.

For the wood stud-7 shown in Figure 3.82, *Paecilomyces variotii* comprised 84% of the total fungal DNA measured. This was the only material that *P. variotii* comprised a majority of the DNA, although this species was also a major contributor to the total fungal DNA found on the wet wood stud. The second species of note for the wet wood stud material was *Chaetomium globosum* which made up another 11% of the total DNA. The *Aspergillus* species were less dominant with *Aspergillus fumigatus* comprising 4.6% of the total DNA. The wet wood stud-7 ranked third overall for the total concentration of all fungal DNA measured.

المنارة للاستشارات
www.manaraa.com

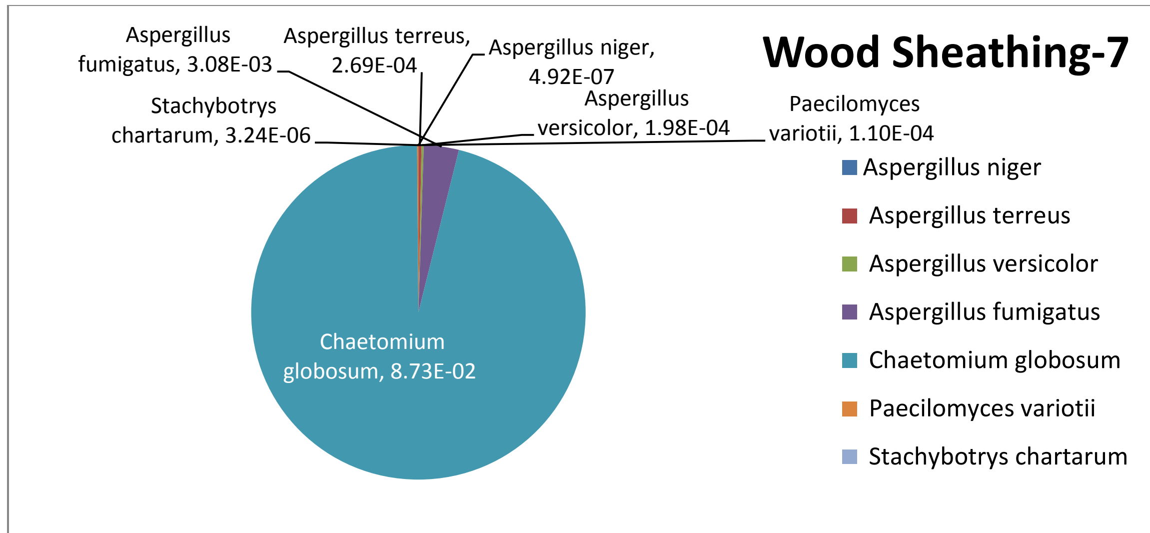

Figure 3.83 The distribution of the selected mold species, based on total DNA concentrations, on wood sheathing

The wood sheathing (Figure 3.83) was dominated by *Chaetomium globosum*, with this species comprising 96% of the total fungal DNA found on this material. *Aspergillus fumigatus* contributed another 3.4% to the total fungal DNA. The wood sheathing supported the highest average concentration for two of the seven fungi measured including *Chaetomium globosum* and *Aspergillus versicolor*. Although *A. versicolor* was found in the highest concentration on the wood sheathing, its overall contribution to the total fungal DNA on the sheathing was less than 1%. The wood sheathing tied for 4th ($2.27E^{-02}$) with gypsum-2 and the dry gypsum for total fungal DNA concentrations.

المنارة للاستشارات
www.manaraa.com

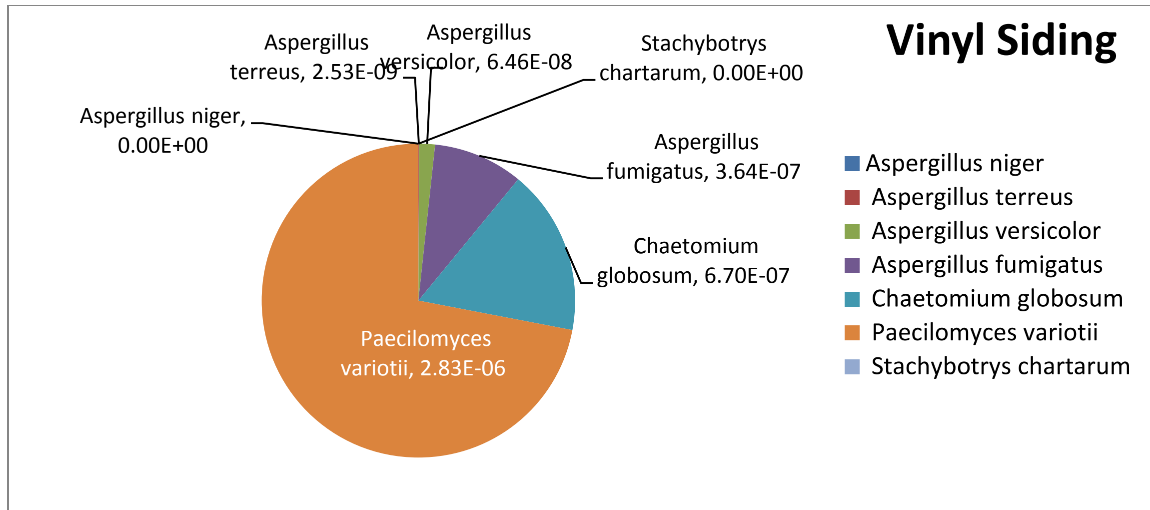

Figure 3.84 The distribution of the selected mold species, based on total DNA concentrations, on vinyl siding.

The vinyl siding supported the lowest total concentration of fungal DNA ranking 11th with only 1.97E-06 total DNA detected. This is not suprising since the vinyl does not provide a good environment for mold development. *Paecilomyes variotii* comprised the majority of fungal DNA contributing 72% to the total. This was followed by *Chaetomium globosum* making up 17% and *Aspergillus fumigatus* with another 9%. Two species were not detected at all on the vinyl siding, *Aspergillus niger* and *Stachybotrys chartarum*.

المنارة للاستشارات
www.manaraa.com

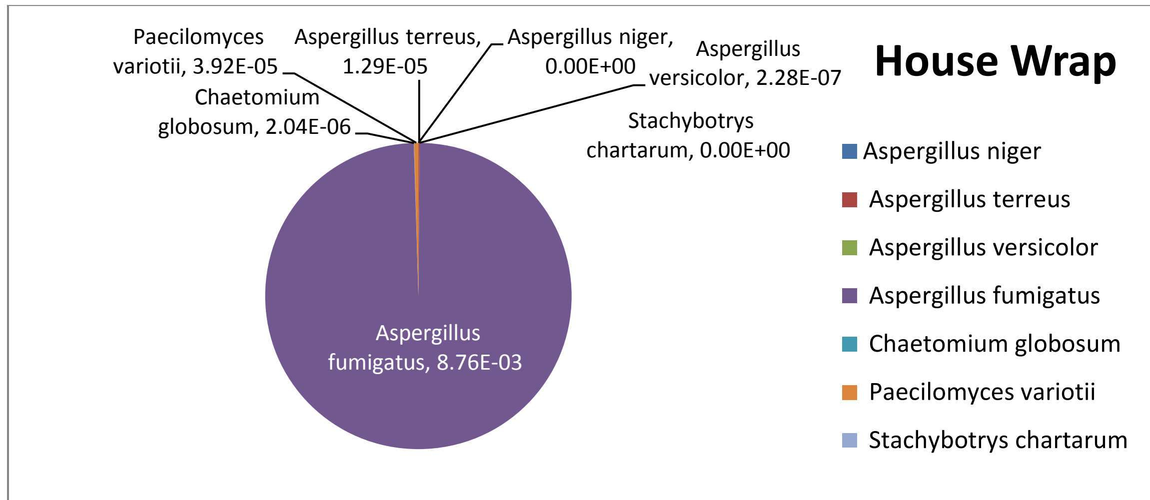

Figure 3.85 The distribution of the selected mold species, based on toal DNA concentrations, on house wrap.

The house wrap supported low concentrations of all mold species and ranked 10 out of 11 for total fungal DNA concentrations (4.67E^{-04}). *Aspergillus fumigatus* was the most prevalent species comprising 92% of the total DNA. *Paecilomyces variotii* made up another 6% of the total fungal DNA for this material. Two species were not detected at all on the house wrap, *Aspergillus niger* and *Stachybotrys chartarum*.

المنارة للاستشارات
www.manaraa.com

Quantization of Mold on Dry Materials

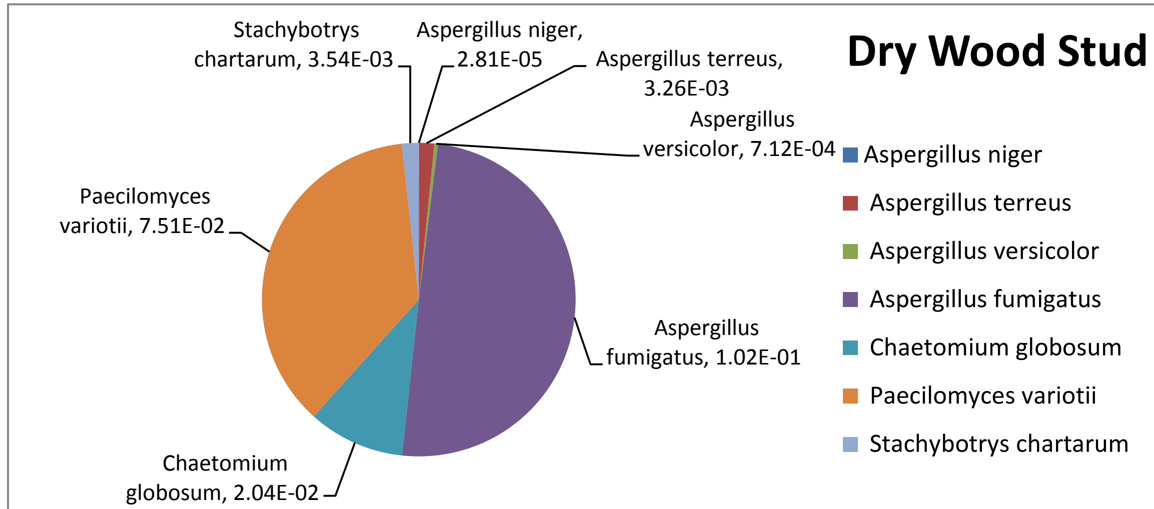

Figure 3.86 The distribution of the selected mold species, based on total DNA concentrations, on the dry wood stud.

The dry wood stud contained high concentrations of fungal DNA ($2.05E^{-01}$) and tied with the batt insulation as supporting the most fungi (tied for #1). The same three species that dominated the wet wood stud also dominated the dry wood stud except in different proportions. The dominant species on the dry wood stud was *Aspergillus fumigatus* comprising 50% of the total DNA, followed by *Paecilomyces variotii* with 36% and *Chaetomium globosum* with 10%. The dry wood stud supported the highest individual or average concentration for five of the seven mold species measured. Only *Chaetomium globosum*, which was higher on the wet wood sheathing, and *Stachybotrys chartarum*, which was higher on the dry gypsum, varied. Obviously the dry wood stud maintained a high level of fungal DNA seven months after flooding.

www.manaraa.com

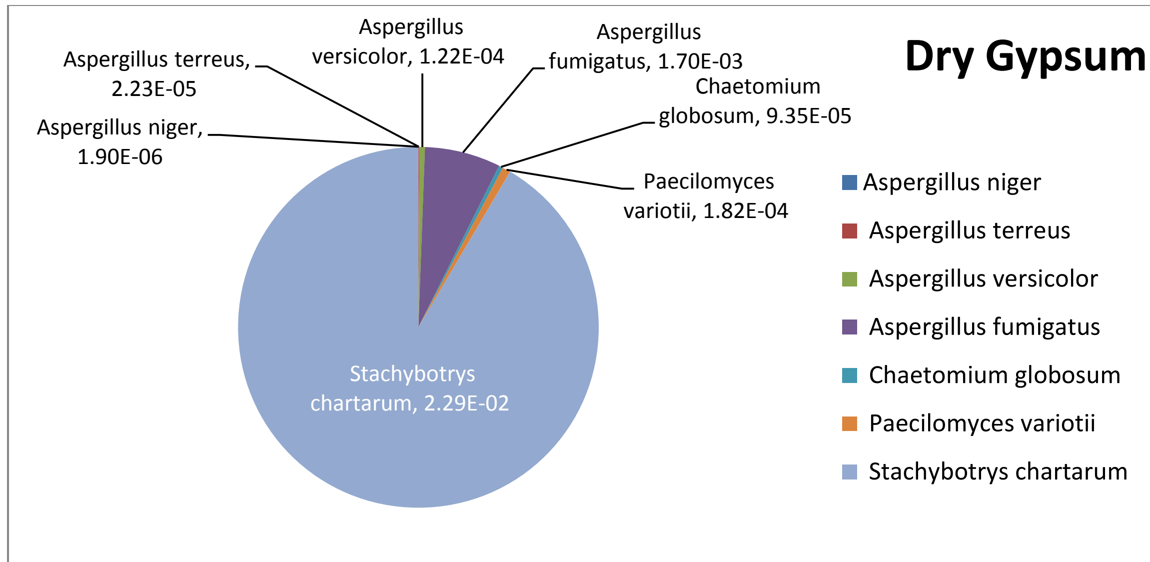

Figure 3.87 The distribution of the selected mold species, based on total DNA concentrations, on the dry gypsum.

The dry gypsum in Figure 3.88 supported the highest concentration of *Stachybotrys chartarum* compared to all other materials tested and this species comprised 92% of the total fungal DNA on this wall material. *Aspergillus fumigatus* contributed another 7% to the total. This material ranked tied for fourth (2.50E^{-02}) in total fungal DNA concentrations with gypsum-2 and the wood sheathing. The broad human health concern over the presence of *Stachybotrys chartarum* within the indoor environment makes its presence in such a notable concentration seven months after flooding a subject for further research.

المنارة للاستشارات
www.manaraa.com

CHAPTER IV

CONCLUSIONS

In this study a total of 168 samples were collected from a flood unit at Tuskegee Univesity where wall building materials were subjected to flood-like conditions similar to Hurricane Katrina. The 'wet' samples included fiberglass batt insulation (R-13), solid wood stud, vinyl siding, Tyvec housewrap, plywood sheathing, and gypsum wallboard. After flooding, the samples were separated to above and below the water line, and sectioned into front, middle, and rear groupings. Seven months after flooding, dry samples which included wood stud and gypsum wallboard were also analyzed.

Identification of Molds

In order to determine the types of molds that could exist within a flooded home, cloning and culturing methods were performed. The different species that were positively identified within the flood unit wall materials included *Aspergillus niger*, *Aspergillus terreus*, *Aspergillus versicolor*, *Aspergillus fumigatus*, *Chaetomium globosum*, *Cladosporium sp., Penicillium chryogenum*, *Pencillium decumbens*, *Penicillium purpurogenum, Trichoderma viride, Trichoderma reesei, Trichoderma harzianum*, *Acremonium alternatum*, *Alternaria alternata*, *Acremonium strictum*, *Fusarium solani, Fusarium oxysporum, Pestalotiopsis maculans*, *Phoma herbarum,* and *Rhizocotonia solani*.

المنارة للاستشارات
www.manaraa.com

The different molds were analyzed for presence on the different types and components of wall materials as well as location above or below the water line. *Acremonium strictum, Aspergillus niger, Chaetomium globosum, Fusarium oxysporum, Trichoderma harzianum, Trichoderma reesei,* and *Trichoderma viride* were all identified both above and below the water line on the wet materials. *Aspergillus fumigatus* and *Stachybotrys chartarum* were identified more frequently than any other type of fungi both above and below the water line on the dry materials.

Quantiation of Molds on Wall Materials

Real-time PCR was used to quantiate the different types of molds that were identified and selected within the flood unit materials. To determine the quantity of each fungus species, EPA primers were used for *Aspergillus fumigatus, Aspergillus niger, Aspergillus terreus, Aspergillus versicolor, Chaetomium globosum, Paecilomyces variotii,* and *Stachybotrys chartarum*.

It was shown that using the primers designed by the EPA proved to be more successful in generating a standard curve using real-time PCR for the individual species that were selected. However, the EPA primers for *Trichoderma virde, Trichoderma reesei*, *Trichoderma harzianum*, *Penicillium purpurogenum, Penicillium decumbens*, and *Penicillium chrysogenum* were unable to produce a successful standard curve. Therefore these molds were not quantitated in this investigation and their absence will skew the results. Additional work will be done to either design new primers or locate alternative primers for these missing species.

المنارة للاستشارات
www.manaraa.com

Summary of Mold Species found on Flooded Wall Materials

Aspergillus fumigatus was found in the highest concentration of any of the seven fungi measured on both the wet and and the dry wall materials. In the wet materials *A. fumigatus* was found at twice the concentration primarily on the batt insulation below the water line and on the dry materials. It was highest on the wood stud. Since this human pathogen was found in such high concentrations on both the wet and dry building materials, individuals should monitor for this mold in flood damaged homes in the southeastern United States.

Paecilomyces variotii was found in the second highest concentration on the damaged building materials after flooding for both the wet and dry materials. The highest concentrations detected were on the wet wood stud (statistically greater than on other wet materials) and the dry wood stud, with the dry stud supporting 1.6x greater levels of this mold. This organism was statistically most likely to be found on the wet materials above the water line compared to below the water line.

The fungus that is considered a cellulolytic species, *Chaetomium globosum* was found in notably high concentrations on the wood materials. This species is present in the third highest concentration on the wet materials and the fourth highest concentration on the dry materials. *C. globosum* was frequently present below the water line compared to above the water line.

Stachybotrys chartarum can be found on water damaged gypsum wallboard and can contain two chemotypes (S and A), which can produce different mycotoxins on wet and dry materials. In this study after flooding, *S. chartarum* was found in high to moderately high concentrations on a few of the wall materials. It was present in the fourth

المنارة للاستشارات
www.manaraa.com

highest concentration on the wet materials particularly on the foil of the batt insulation and on the paper of gypsum-2; and was present in the third highest concentration on the dry materials. Although sporadic in its occurrence, the presence of *Stachybotrys chartarum* both on the wet and dry materials is a concern.

Aspergillus terreus is considered uncommon on building materials however, *A. terreus* was detected on most wet and dry materials after flooding. It was the fifth most common mold species encountered in this study on both the wet and dry materials. *A. terreus* was found in statistically higher concentrations on the batt insulation above the water line and wood sheathing below the water line. It is worth noting that the batt and the wood sheathing came from the same wall unit and would have been close to each other. It was surprising to find, based on the findings that *A. terreus* remained in high concentrations on the dry materials seven months after flooding.

The fungal species that is common in indoor environments is *Aspergillus versicolor*. This species was the sixth most common species encountered in this study on both wet and dry materials. It was detected both above and below the water on the different building materials where the highest concentrations were found on the wet wood sheathing and the dry wood stud. *A. versicolor* wasn't found in any particular location and it appeared to survive on the dry materials seven months after flooding.

The concentrations of *Aspergillus niger* that were found on both the wet and dry materials were very low compared to the other mold species. Typically this organism is not associated with contaminated building materials, however when comparing the concentrations of wet and dry, the dry wood stud supported the highest average and the highest individual concentration. *Aspergillus niger* was found in a statistically higher

المنارة للاستشارات
www.manaraa.com

concentration on the batt insulation compared to the other wet building materials. It appears; *A. niger* was statistically more likely to be found above the water line than below the water line, which suggests that it grows well in environments when lower moisture is present.

Summary of Flooded Wall Materials

The fiberglass batt insulation supported the highest concentration of total mold compared to all other wet wall materials. The two mold species of particular note found on the batt were *Aspergillus fumigatus* and *Stachybotrys chartarum*. A majority of the fungi were found above the water line, the exception being *A. fumigatus*. An unfortunate consequence in this study was to not have sampled the batt insulation seven months after flooding. The batt could potentially serve as a reservoir for the adjacent wall materials as well as a source of inoculum for the dry materials sampled. These concerns certainly warrant further investigation.

The southern yellow pine wood stud supported the second highest concentration of mold for the wet materials and the highest concentration of molds for the dry materials. Based on the observations made during culturing, this result is surprising. Different mold species (*Paecilomyces variotii*) dominated the wet wood stud compared to the other wet materials. The *Aspergillus* species were actually present in similar concentrations to the average wet gypsum, but were minimized by the levels of *P. variotii* and *C. globosum*. The same three species that were most common on the wet wood stud were also most common on the dry wood stud, however their proportions shifted. *Aspergillus fumigatus* comprised a majority of the molds on the dry wood stud, followed by *P. variotii* and *C. globosum*. It is also surprising to find such high concentrations of

المنارة للاستشارات
www.manaraa.com

mold on the wood stud seven months after flooding. Since a wood stud is not a wall component likely to be replaced after a flood event, good remediation of this wall material is essential.

The plywood sheathing supported the third highest concentration of mold when comparing all wet wall materials. This wall material was dominated by *Chaetomium globosum*. It should be noted that the three wall materials which supported the highest concentration of molds comprise the interior of a wall. How the mold and the batt insulation impacts the mold on the wood stud or wood sheathing or vice versa and do these materials serve as sources of inoculum after recovery warrants further investigations.

The gypsum wallboards were the only materials sampled from more than one location within the flood unit. Of the four wet gypsum boards analyzed, gypsum-2 stood out as very different from the other three boards. It is not known exactly where gypsum-2 came from within the flood unit nor did it show the presence of a flood water line like the other wallboards. Gypsum-2 was dominated by two mold species (*Chaetomium globosum* and *Stachybotrys chartarum*) not found in any notable concentrations on the other three gypsum boards. If this sample was removed from the averages of mold on the other gypsum wallboards, 99.5% of all mold detected on gypsum would be *Aspergillus* species, with *A. fumigatus* comprising the majority. Overall, gypsum ranked fourth in mold concentrations on wet materials. The dry gypsum contained high levels of mold and was dominated by *Stachybotrys chartarum*. The broad human health concern over the presence of *Stachybotrys chartarum* within the indoor environment makes its presence in such a notable concentration seven months after flooding a subject for further research.

المنارة للاستشارات
www.manaraa.com

The last two wall materials, house wrap and vinyl siding, supported the lowest concentration of molds. The house wrap did contain moderate levels of *A. fumigatus*, however, it is likely these two materials contribute little to the overall mold population within a flooded wall.

To further investigate the impacts of fungal communities on flooded building materials, the types of mold species that could exist after flooding are now known, thus an investigation into these types of molds can now be studied in depth. These studies could include mycotoxin development or newer remediation methods to prevent mold growth and make indoor environments safer for humans after flooding.

المنارة للاستشارات
www.manaraa.com

REFERENCES

Allsopp D. 1985. Biology and growth requirements of moulds and other deteriogenic fungi. Journal Of The Society Of Archivists 7, 530.

Ammann, H.M. 2003. Is Indoor Mold Contamination a Threat to Health? Part Two. Journal of Environmental Health 66, 47-49.

Andersen, B., Frisvad, J.C., Sondergaard, I., Rasmussen, S., Larsen, L. 2011. Associations between Fungal Species and Water-Damaged Building Materials. APPLIED AND ENVIRONMENTAL MICROBIOLOGY 77, 4180-4188.

Ashley, N. A., Valsaraj, K. T., Thibodeaux, L. J. 2009. Multiphase Contaminant Distributions Inside Flooded Homes in New Orleans, Louisiana, After Hurricane Katrina: A Modeling Study. Environmental Engineering Science 26, 609-619.

Bellanger, A.P., Reboux, G., Roussel, S., Grenouillet, F., Didier-Scherer, E., Dalphin, J.C., and Millon, L. 2009. Indoor fungal contamination of moisture-damaged and allergic patient housing analyzed using real-time PCR. Letters in Applied Microbiology 49, 260-266.

The Bible, Leviticus 14th chapter, verses 34-45.

Bloom, E., Grimsley, L.F., 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.

Brody, S. D., Zahran, S., Maghelal, P., Grover, H., Highfield, W.E. 2007. The Rising Costs of Floods. Journal of the American Planning Association 73, 330-345.

Brody, S. D., Zahran, S., Highfield, W.E., Grover, H., Vedlitz A. 2008. Identifying the impact of the Built Environment on flood damage in Texas. Disasters 1, 1-18.

Bush, R.K., Portnoy, J.M., Saxon, A., Terr, A.I., Wood, R.A. 2006. The medical effects of mold exposure. Journal of Allergy and Clinical Immunology 2, 326-333.

Chew, G.L., Wilson, J., Rabito, F.A., Grimsley, F., Iqbal, S., Reponen, T., Muilenberg, M.L., Thorne, P.S., Dearborn, D.G., Morley, R.L. 2006. Mold and Endotoxin Levels in the Aftermath of Hurricane Katrina: A Pilot Project of Homes in New Orleans Undergoing Renovation. Environmental Health Perspectives 114, 1883-1889.

المنارة للاستشارات
www.manaraa.com

Chiocchio, V. M., Matković, L. 2011. DETERMINATION OF ERGOSTEROL IN CELLULAR FUNGI BY HPLC. A MODIFIED TECHNIQUE. The Journal of the Argentine Chemical Society 98, 10-15.

Curtis, L., Lieberman, A., Stark, M.,Rea, W., Vetter, M. 2004. Adverse Health Effects of Indoor Molds. Journal of Nutritional & Environmental Medicine 3, 261-74.

Dantigny, P., Bensoussan, M., Vasseur, V., Lebrihi, A., Buchet, C., Ismaili-Alaoui, M., Devlieghere, F., Roussos, S. 2006. Standardisation of methods for assessing mould germination: A workshop report. International Journal of Food Microbiology 2, 286-291.

Dillion, H.K., Miller, J.D., Sorenson, W.G., Douwes, J., Jacobs, R.R. 1999. Review of Methods Applicable to the Assessment of Mold Exposure to Children. Environmental Health Perspectives 3, 473-480.

Du, W., FitzGerald, G.J., Clark, M., Hou, X.Y. 2010. Health impacts of floods. Prehospital and Disaster Med 3, 265–272.

Dumon, H., Palot, A., Charpin-Kadouch, C. 2009. Mold Species identified in flood dwellings. Aerobiologia 25, 341-344.

Engelhart, S., Loock, A., Skutlarek, D., Sagunski, H., Lommel, A., Färber, H., Exner, M. 2002. Occurrence of toxigenic *Aspergillus versicolor* isolates and sterigmatocystin in carpet dust from damp indoor environments. Applied and Environmental Microbiology 8, 3886-3890.

Flannigan, B., Samson, R.A., Miller, J.D. 2011. Microorganisms in Home and Indoor Work Environments. Diversity, Health Impacts, Investigation and Control, 2nd ed

Fisher, G., Dott, W. 2003. Relevance of airborne fungi and their secondary metabolites for environmental, occupational and indoor hygiene. Archives of Microbiology 179, 75-82.

Gorny, R.L., Reponen, T., Grinshpun, S. A., Willeke, K. 2001. Source strength of fungal spore aerosolization from moldy building. Atmospheric Environment 28, 4853-4862.

Gover, D.W., 1999. DISPERSAL OF FUNGAL SPORES. Available from http://www.cfr.washington.edu/classes.esrm.444/Reading%20files/Spore%20dispersal.pdf

Hardin, B.D. 2003. Adverse Human Health Effects Associated with Molds in the Indoor Environment. Journal of Occupational and Environmental Medicine 5, 470-477.

المنارة للاستشارات
www.manaraa.com

Jarvis, J. Q., Morey, P. R. 2001. Allergic Respiratory Disease and Fungal Remediation in a Building in a Subtropical Climate. Applied Occupational & Environmental Hygiene. 16, 380-388.

Jonkman, S. N., Kelman, I. 2005. An Analysis of the causes and Circumstances of Flood Disaster Deaths. Disasters 1, 75-97.

Koskinen, O.M., Husman, T.M., Meklin, T.M., Nevalainen, A.I. 1999. The relationship between moisture or mould observation in houses and the state of health of their occupants. European Respiratory Journal 14, 1363-1367.

Landeweert, R., Leeflang, P., Kuyper, T. W., Hoffland, E., Rosling, A., Wernars, K., & Smit, E. 2003. Molecular identification of ectomycorrhizal mycelium in soil horizons. Applied and Environmental Microbiology 1, 327-333.

Latgé, J. P. 1999. *Aspergillus fumigatus* and aspergillosis. Clinical microbiology reviews 2, 310-350.

Lievens, B., Brouwer, M., Vanachter, A. C., Cammue, B., Thomma, B. P. 2006. Real-time PCR for detection and quantification of fungal and oomycete tomato pathogens in plant and soil samples. Plant Science 1, 155-165.

Lstiburek, J. W. 2007. The Material View of Mold. American Society of Heating, Refrigerating and Air Conditioning Engineers 49, 61-63.

Lstiburek, J. W. 2009. 5 Fundamental Changes In the Last 50 Years. American Society of Heating, Refrigerating and Air Conditioning Engineers 51, 52-56.

Mehta, M., Scarborough, W., Armpriest, D. 2008. Plywood Panels. Pp. 255-258. In: W. Scarborough and Diane Armpriest (eds.), Building Construction: Principles, Materials, and Systems.Pearson Education, Inc. Upper Saddle River, NJ

Mehta, M., Scarborough, W., Armpriest, D. 2008. Vinyl Siding. Pp. 317. Building Construction: Principles, Materials, and Systems.Pearson Education, Inc. Upper Saddle River, NJ

Mehta, M., Scarborough, W., Armpriest, D. 2008. Gypsum Board. Pp 320-323. Building Construction: Principles, Materials, and Systems.Pearson Education, Inc. Upper Saddle River, NJ

Mehta, M., Scarborough, W., Armpriest, D. 2008. Fibrous Insulation. Pp 123-124. Building Construction: Principles, Materials, and Systems.Pearson Education, Inc. Upper Saddle River, NJ

Menetrez, M.Y., Foarde, K.K., Esch, R.K., Schwartz, T.D., Dean, T.R., Hays, M.D., Cho, S.H., Betancourt, D.A., Moore, S.A. 2009. An evaluation of indoor and outdoor biological particulate matter. Atmospheric Environment 34, 5476-5483

المنارة للاستشارات
www.manaraa.com

Mullis, K. B., Faloona, F. A., Scharf, S. J., Saiki, R. K., Horn, G. T., Erlich, H. 1986. Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. In Cold Spring Harb Symp Quant Biol 51, 263-273.

Nielsen, K.F., Gravesen, S., Nielsen, P.A., Andersen, B., Thrane, U., Frisvad, J.C. 1999. Production of mycotoxins on artificially and naturally infested building materials. Mycopathologia 145, 43-56.

Parker, H. 1958. Wood. Pp. 50- 67. In:J.W. Macguire and C.M. Gay (eds.), Materials and Methods of Architectural Construction. Third Edition. John wiley and sons

Pearce, M., Huelman, P.H., Janni, K.A., Olsen, W., Seavey, R.T., Velsey, D. 1995. Long-term monitoring of mold contamination in flooded homes. Journal of Environmental Health 58, 6-11.

Pirages, S.W. 2005. Mold and Health Issues- Facts Versus Fiction Engineered Systems 10, 12-15.

Portney, J.M., Barnes C. S., Kennedy, K. 2004. Sampling for Indoor Fungi. Journal of Allergy and Clinical Immunology 2, 189-198.

Portney, J.M., Kwak, K., Dowling, P., VanOsdol, T., Barnes, C. 2005. Health effects of indoor fungi. Annals of Allergy, Asthma and Immunology 94, 313-320.

Rao, C.Y., Riggs, M.A., Chew, G.L., Muilenberg, M.L., Thorne, P.S., Sicke, V. D., Dunn, K.H., Brown, C. 2007. Characterization of Airborne Molds, Endotoxins, and Glucans in Homes in New Orleans after Hurricanes Katrina and Rita. Applied and Environmental Microbiology 73, 1630–1634.

Reeslev, M., Miller, M., Nielsen, K.F. 2003. Quantifying Mold Biomass on Gypsum Board: Comparison of Ergosterol and Beta-N-Acetylhexosaminidase as Mold Biomass Parameters. Applied and Environmental Microbiology 69, 3996-3998.

Ren, P., Ahearn, D.G., Crow, S.A. 1998. Mycotoxins of *Alternaria alternata* produced on ceiling tiles. Journal of Industrial Microbiology 50, 188-189.

Reponen, T., Seo, S. C., Grimsley, F., Lee, T., Crawford, C., & Grinshpun, S. A. 2007. Fungal fragments in moldy houses: a field study in homes in New Orleans and Southern Ohio. Atmospheric Environment 37, 8140-8149.

Rylander, R. 1998. Microbial Cell Wall Constituents in Indoor Air and their Relation to Disease. Indoor Air 4, 59-65.

Samson, R.A., Houbraken, J., Thrane. U., Frisvad, J.C., Anderson, B. 2010. Food and indoor fungi. CBS-Knaw Fungal Biodiversity Centre Utrecht. The Netherlands. 377 pages

المنارة للاستشارات
www.manaraa.com

Simmons, L.H. 2001. Gypsum Board Systems. Pp. 719-724: J.L Schmidt and W.H. Lewis (eds.), Construction: Principles, Materials, and Methods. Seventh Edition. John Wiley and Sons

Simmons, L. H. 2001. Conventional Framing and Furring. Pp. 412-424: J.L. Schmidt and W.H. Lewis (eds.), Construction:Principles, Materials, and Methods. Seventh Edition. John Wiley and Sons

Simmons, L.H. 2001. Building Insulation. Pp. 499-502: J.L. Schmidt and W.H. Lewis (eds.), Construction: Principles, Materials, and Methods. Seventh Edition. John Wiley and Sons.

Simmons, L.H. 2001. Vinyl Siding. Pp. 583-585: J.L. Schmidt and W. H. Lewis (eds)., Construction: Principles, Materials, and Methods. Seventh Edition. John Wiley and Sons.

Singh, J., Yu, C. 2010. Building Pathology- Toxic Mold Remediation. SHB2010 - 3rd International Symposium on Sustainable Healthy Buildings.

Sobek, E. A. 2007. DNA FORENSIC MOLD DETECTION© Technical Brief.

Straube, J. 2006. Moisture and Materials. Building Science Digest 138, 1-7.

Vesper, S. 2011. Traditional mould analysis compared to a DNA-based method of mould analysis. Critical Reviews in Microbiology 37, 15-24.

Viitanen, H., Ojanen, T. 2007. Improved Model to Predict Mould Growth in Building Materials, In: Thermal Performance of the Exterior Envelopes of Whole Buildings X Proceedings CD, 2–7 December, Clearwater Beach, USA, ASHRAE, DOE, ORNL.

Wong, M. L., Medrano, J. F. 2005. Real-time PCR for mRNA quantitation. Biotechniques 1, 75.

المنارة للاستشارات
www.manaraa.com