ENVIRONMENTAL FACTORS INFLUENCING FUNGAL GROWTH ON GYPSUM BOARDS AND THEIR BIODEGRADATION: A UNIVERSITY CAMPUS CASE STUDY

by

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Abstract

Contamination and growth of fungi within buildings, has gained increasing public attention within the last decade. Indoor fungal growth can have many adverse effects on building materials and has been linked to many health effects. In an effort to better understand the different environmental conditions that lead to fungal growth on indoor materials of drywall, and observe the biodegradative capabilities of these organisms, a case study was conducted at the University of British Columbia-Okanagan campus.

The results of this study showed that age, and type of rooms can affect the diversity and composition of fungal taxa, with rooms in older buildings and laboratories supporting a higher diversity of fungi. The type of flooring and presence of carpet was not an influencing factor on fungal growth and diversity observed on drywall samples. A higher degree of fungal growth also affected the physical and mechanical properties of these building materials, with more fungi increasing the dry weight loss and decreasing the tensile strength of drywall. Although there are several epidemiological studies on the association between indoor fungi and the development of health problems, much still needs to be learned about what factors in indoor environments lead to the growth of these microorganisms. With a better understanding of which factors and environmental conditions trigger fungal growth in built environments, we can eventually better design indoor living spaces occupied by humans and develop methods for dealing with such problems.

Preface

This thesis presents an experimental-statistical analysis of the effects of fungal growth on gypsum boards. This research was conducted at the University of British Columbia, Okanagan campus, under co-supervision of Dr. John Klironomos and Dr. Abbas S. Milani. A preliminary version of this work and a later completed form of parts of this thesis were published in the 1st and 2nd Annual Biology Graduate Symposium, Biology Department, UBC Okanagan, 2015 & 2016. A version of chapters 2-5 has been submitted in a journal for publication. This work was drafted in whole by N. Kazemian with editorial comments by Drs. J. Klironomos, A. S. Milani, and L. Nelson.

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Dedication

This thesis is dedicated to my loving parents:

Fahimeh Tajzadeh & Hossein Kazemian

Chapter 1. Background and Thesis Organization

1.1 Introduction

In recent years, there has been a growing global concern regarding the potential impacts of the environment on health and human development. Over time humans have evolved to live in confined spaces known as built environments. Presently, people spend 80-95% of their daily lives indoors [Cabral 2010; Decarro et al. 2003], starting from childhood in daycares and schools, to work environments and homes. Even leisure time is most often spent in indoor environments. As a result of this, the exposure to airborne substances has become an important factor to measure and investigate in indoor environments.

The U.S. Environmental Protection Agency reports that indoor air can be two to five times more polluted than outdoor air [Portnoy et al. 2004]. Indoor environments are often composed of a very complex ecosystem that contains viable and dead organisms as well as allergens, small fragments of organisms, and metabolites such as volatile organic compounds and mycotoxins [Block 1953; Cabral 2010; Lee et al. 2010]. Saprotrophic fungi are among the many microorganisms found in indoor environments, and are key agents of structural decay [Scott et al. 2004]. These fungi can utilize carbon and nutrients, and acquire nutrients via breakdown of starch, cellulose, and lignin [Hoang et al. 2010]. Fungal spores in the air are impossible to eliminate in indoor environments; however, controlling humidity and temperature is the best strategy for reducing growth of these microorganisms [Mihinova & Pieckova 2012; Vacher et al. 2010]. Moisture can enter indoor environments through many different sources such as leakages, floods, increased condensation, and even activities of occupants in spaces [Adan & Samson 2011; Mihinova & Pieckova 2012]. Other factors such as age of buildings [Sivasubramini et al. 2004], number of occupants [Sharpe et al. 2014], and flooring, such as carpets [Wani et al. 2014], can also affect the concentration of fungal spores found and released in indoor environments.

Fungi in built environments can grow on the surface of many indoor materials such as drywall, ceiling tiles, carpets, insulation material, etc. [Genuis 2007]. Drywall (or gypsum board), is a ubiquitous building material used in all construction. This indoor material is made of gypsum plaster and two thick sheets of paper [Wakili et al. 2014]. While drywall has many advantages, it is also highly susceptible to microbial growth due to the cellulosic paper backing [Pasanen et al. 1997]. The degrading enzymes and metabolites produced by microorganisms induce hydrolysis of cellulose macromolecules, leading to discoloration and an increase in damage of internal areas of the material [Webb et al. 2000; Pekhtasheva et al. 2012]**.** Thus, fungal growth can degrade this material and affect the structural integrity of a building [Portnoy et al. 2004].

Concern over contamination and growth of fungi within buildings has gained increasing public attention, due to the increasing prevalence of mould-related health problems [Nielsen 2003; Mihinova & Pieckova 2012; Wani et al. 2014]. Fungal growth in homes, institutions, and work places has been reported in many publications, including the World Health Organization guidelines: *dampness and moulds,* which concluded that there is sufficient evidence that occupants in mouldy buildings are at increasing risk of respiratory problems, respiratory infections, allergic diseases, and asthma [Andersen et al. 2002; Cabral 2010; Douwes et al. 2003]. Thus, human exposure assessment and environmental evaluations are increasingly becoming recognized as essential components of building design and health care of patients [Genuis 2007]. Therefore, it is important to better understand the diversity and composition of indoor fungal species that are present due to different environmental factors, and to explore their contribution to degradation of indoor material and to occupants' health problems.

1.2 Motivation of this research

There have been several attempts to investigate the fungal community of indoor environments and on building materials such as gypsum drywall [Andersen et al. 2011; Nielsen 2002; Nielsen et al. 2004]; however, most of these studies focus on the mycotoxins produced [Nielsen et al. 2004; Nielsen et al. 1998], the finishes applied (paint and wallpaper) [Krause et al. 2006; Vacher et al. 2010], or the abundance of cultural or pathogenic strains and their health effects [Andersen et al. 2002], rather than a survey of fungal diversity in indoor spaces; hence, the environmental factors that determine diversity and composition of the fungal community in built environments are still poorly understood and require further investigation. Although many models exist to assess indoor environments, no one model or tool is predictive of the whole environment [Keall et al. 2012; Vesper et al. 2007]. Therefore, further environmental features that affect fungal growth need to be examined. With today's building materials and design, indoor environments are more prone to fungal growth. Fungal spores available in the air, along with the humidity from the environment may lead to growth on many indoor materials. Fungal growth on a building material can affect the structural integrity of that material, as the fungus can use it as a nutrient source and has the ability to degrade it over time [Portnoy et al. 2004]. Exposure to mould and dampness is also known to have many health effects and is thus a significant aspect for further study [Andersen et al. 2002]. Accordingly, the overall scope of the proposed research was to better understand the different environmental conditions that lead to fungal growth and to link fungal community composition of indoor dust to biodegradation and biodeterioration of drywall as a representative example of building materials.

1.3 Objectives

The association between fungal growth in indoor environments and the development of health problems, as well as fungal effects on indoor materials has been established by some

epidemiological studies [Genuis 2007; Miller & McMullin 2014; Nielsen et al. 1998; Cox-Ganser 2015]. Research is still needed, however, to learn which environmental factors in indoor environments lead to fungal growth on common building materials and cause their biodegradation, along with potential health risks for occupants. With a better understanding of such factors, builders can further optimize the design of indoor living spaces occupied by humans and develop methods for dealing with such problems. To address parts of the above gap, the main aim of this thesis has been to identify factors leading to indoor fungal growth on gypsum drywall, and to determine their effects on physical and mechanical properties of this indoor material by conducting a case study on select buildings at the UBC-Okanagan. The specific objectives (research questions) are as follows.

Objective 1: Evaluate different building attributes and environmental conditions across the campus that may influence fungal growth on drywall;

Control factors chosen:

a) Age of building

b) Different function/types of rooms

c) Type of flooring

Random effects chosen (i.e., other uncontrolled factors which may vary within and between rooms) [Ramos & Stephens 2014]:

a) Room temperature

b) Room humidity

c) Number of occupants

d) Presence of windows

e) Cleanliness level

Objective 2: Determine which building attributes would potentially lead to differences in the composition of fungal growth on drywall.

Objective 3: Detect and observe the growth of select health-problematic fungal species that are known to be human pathogens.

Objective 4: Examine the effects of fungal growth on the physical and mechanical properties of drywall.

1.4 Thesis outline

Chapter 1 describes the objectives and motivation of the study. Chapter 2 includes a literature review of the factors affecting fungal growth in built environments, where a number of earlier studies are discussed and compared. Chapter 3 provides the methodology used in this study to fulfil the objectives defined in section 1.3. Chapter 4 presents and discusses the results obtained for the tests, both biological tests and mechanical/materials tests. A discussion of the results and data analysis is also included in this chapter. Finally, chapter 5 includes the main conclusions of the study and outlines potential future work.

Chapter 2. Literature Review

Built environments are complex ecosystems that support a high diversity of microbial life including human pathogens and commensals [Kembel et al. 2012]. People spend the majority of their daily lives indoors [Cabral 2010; Decarro et al. 2003] and are constantly inhaling the available air in these environments. This chapter includes a literature review of past research on factors affecting fungal growth on many indoor materials, such as gypsum boards.

2.1 Mycobiota of indoor environment

The air we breathe and the environment we live in are profound determinants of our health and well-being. In general, the indoor microbiome consists of both autotrophic (e.g., algae), and heterotrophic (e.g., bacteria, fungi, and protozoans) organisms [Pitkaranta 2012]. High moisture exposure [Mendell et al. 2011], can lead to microbial growth on many indoor materials, leading to low indoor air quality, and subsequently to serious health problems [Cabral 2010]. The main sources of indoor microbes are: i) outside air, ii) indoor surfaces, iii) bodies of humans, and iv) other organisms living indoors [Pakarinen et al. 2008]. Overall there is still a poor understanding of what sources are most significant in shaping the built environment microbiome. In this project, as a case study, the focus will be on the fungal component of indoor environments in various university buildings.

2.1.1 Fungi

Fungi are commonly found in the built environment and have been estimated as one fourth of the earth's total biomass [Scott 2001; Wani et al. 2014]. Fungi are a member of a large group of eukaryotic, heterotrophic organisms that with the presence of water, grow, elongate, and form tube-like structures called hyphae [Genuis 2007], and reproduce by means of spores (tiny highly characteristic and specialized reproductive structures) [Kendrick 1985]. Although fungi are important in ecosystems [Andersen et al. 2011], they may also be problematic in indoor environments. Fungi that live in buildings are saprotophs and are key agents in structural decay. These fungi acquire nutrients via breakdown of starch, cellulose, and lignin by the production of extracellular enzymes [Hoang et al. 2010]. Some indoor fungi may also act as human parasites (e.g., *Aspergillus fumigatus*) [Flannigan et al. 2001; Scott 2001].

Fungi found indoors are classified into two groups, yeast and filamentous fungi. Studies demonstrated that yeast are abundant in houses with pre-dominant species such as *Rhodotorula minuta* and *R. albidus,* which can cause allergic reactions [Verhoeff et al. 1994]. To date, 100-150 species of fungi have been detected in indoor environments, which is only a fraction of the 100,000 discovered [Adan & Samson 2011]. Five hundred of these 100,000 species have also been found to be human pathogens [Anderen et al. 2002].

Indoor microbiota can be categorized into three groups based on the formation of spores: i) airborne conidia produced as dry single spores or in chains from *Aspergillus, Penicillium, Alternaria, Cladosporium;* ii) spores produced in wet slimy heads that don't become easily airborne since they stick together in wet heads, belonging to *Stachybotrys, Acremonium, Fusarium,* and *Trichoderma;* iii)) spores formed in fruiting bodies such as *Phoma* and *Eurotium* [Adan & Samson 2011].

2.2 Indoor fungal growth

The concern for mould growth in buildings is a continuing problem, which is exacerbated due to poor planning, accelerated building processes, and complex building constructions [Adan & Samson 2011; Cabral 2010]. The fraction of buildings with mould contamination in the United States and Canada is about 36% [Sivasubramini et al. 2004]. A home environmental survey by O'Connor et al. (2004), measured the concentration of airborne fungi inside and outside homes and found that indoor fungal concentration was an indicator of the relative mouldiness of a home. The concentration of outdoor bioaerosols can change seasonally and increase in the months of winter (Mihinova & Pieckova 2012). Aerosols reach indoor environments through natural and or mechanical ventilation via windows and doors, and can also be transported by people and animals [Muilenberg 1995]. The concentration of fungal spores in bioaerosols depends on three biological factors. The first is the magnitude of sporulation, which is highest at an optimum temperature of 25–30 °C. The second is the spore release from conidiophores, which is influenced by humidity and air currents. Lastly spore dimensions and weight, which affect how spores can adhere to a surface and enable the germination of fungi. The most abundant fungi in the atmosphere (*Cladosporium*, *Penicillium*, and *Aspergillus*) produce high numbers of small and light spores, and therefore they dominate most environments. *Alternaria* and *Stachybotrys* produce fewer, bigger, and heavier spores, which tend to settle faster. In bioaerosols, these spores live shorter than small and lighter spores [Cabral 2010]. Little is still known about the dynamics of biological aerosols in indoor environments, including fungal spores and, thus, needs further investigation.

2.2.1 Humidity and temperature effects

It is impossible to eliminate all fungal spores in indoor environments; however, moisture control is the best strategy for reducing growth [Mihinova & Pieckova 2012; Cabral 2010].]. Water can enter buildings as both a liquid and gas (water vapour) [**Figure 2.1**]. Once the water comes in contact with the building materials, it can be drawn in via pores or capillaries by a process called absorption, so it is very important to assess moisture in indoor environments [Straube 2006]. The main sources of moisture in indoor environments are via: a) capillary action of building subsoil and groundwater intrusion, b) building design and or operational issues such as leaking, c) raining on buildings, d) condensation, and e) activities of occupants such as in the

bathroom, kitchen, laundry, and spills [Adan & Samson 2011; Mihinova & Pieckova 2012]. Of all the factors that have been studied which affect fungal growth, water has been proven to be a major determinant, along with temperature and time [Vacher et al. 2010]. As a result, flooded areas tend to experience significant fungal exposure. The Centre for Disease Control noted that mould growth will develop on materials that remain wet for 48-72 hours [Genuis 2007].

Fungi that grow on particular material are known as 'associated' fungi and categorized into three groups based on their water activity a_w (a_w x 100=% relative humidity at equilibrium) [Andersen et al. 2002]. These categories consist of: i) primary colonizers that are storage moulds which grow at a_w of less than 0.8 (low moisture level in material), such as *Aspergillus versicolor* (at 12^0 C), and less frequently *A. fumigatus, A. niger, Penicillium palitans, Paecilomyces variotii, and Wallemia seb;* ii) secondary colonizers that require a minimum a_w of 0.8-0.9 (intermediate moisture level) including *Alternaria, Cladosporium, Phoma, Ulocladium*, *A. fumigatus* and *A. versicolor* (at 25^0 C); and iii) tertiary colonizers known as water damage moulds that require a_w of at least 0.9 or higher (high moisture level), such as *Chaetomium globosum, Stachybotrys chartarum,* and *Trichoderma* [Ababutain 2013; DEHS 2010; Flannigan et al. 2001; Grant et al. 1989].

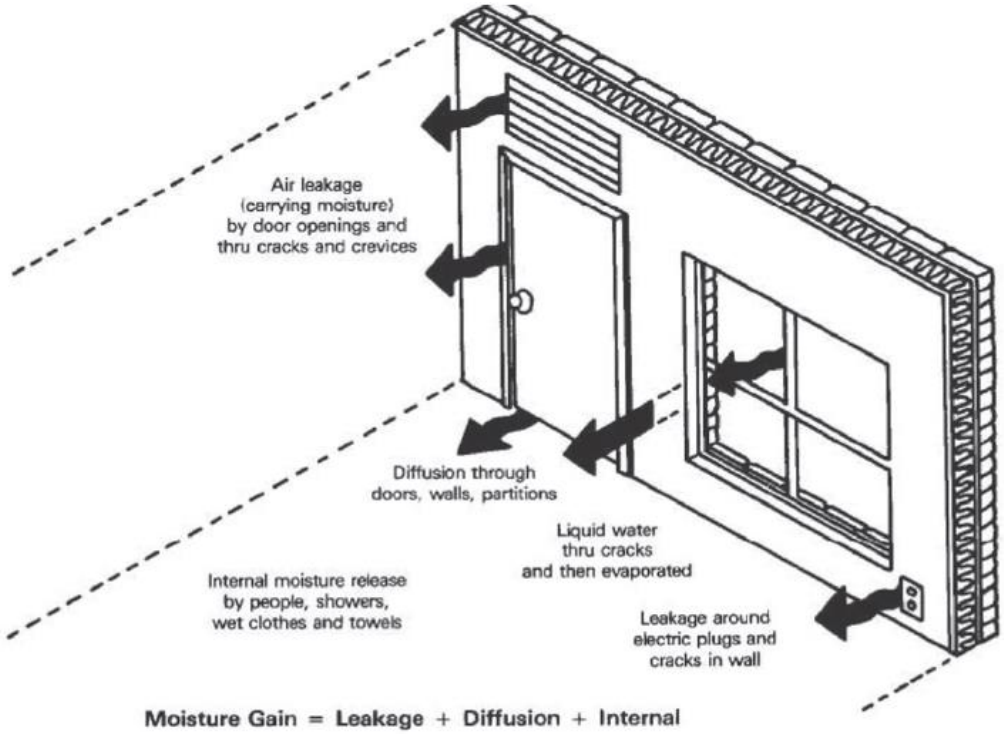


Figure 2.1 Moisture production and entrance in buildings [Mihinova & Pieckova 2012].

It is worth noting that the distribution of moisture is not always uniform and therefore different parts of a medium can lead to varying amounts and different types of fungal growth. For example, the hydrophilic mould *Stachybotrys chartarum* was detected on an extremely wet

surface of a wall, while *A. versicolor* was detected on the drier parts of the growing region [Grant et al. 1989]. All species in indoor environments have their own individual growth requirements and identifying the fungi to the species level could be a good indication of the building condition as a whole [Adan & Samson 2011]. In sub-sections of section 2.3, fungal growth on the most common building materials will be discussed.

2.2.2 Age of buildings

Different environments will support different fungal communities. The age of the building is an important factor affecting the fungal spore concentration in indoor air [Sivasubramini et al. 2004]. Older properties usually have elevated levels of dampness and fungal contamination [Sharpe et al. 2014]. This may be due to a number of factors: i) they are less insulated from the outside and therefore are less efficient at preventing water entrance, ii) they hold higher moisture levels in the building fabric due to older construction materials and techniques, etc. [Sharpe et al. 2014].

2.2.3 Number of occupants

High occupant places also have a higher fungal spore or fragment concentration. The number of people occupying an environment can increase the risk of condensation and proliferation of fungi [Sharpe et al. 2014]. Fungal spore release is affected by the air velocity above the surface and texture of the surface [Andersen et al. 2011]. Kildeso et al. (2003) studied the release of particles from several typical indoor fungi growing on building material (gypsum boards) for 4–6 weeks and saw differences between different fungal species and whether they released spores or fragments. The type of released particles was influenced by the velocity of the air current. However, toxic fungi (*Stachybotrys* sp. and *Chaetomium* sp.) do not readily become airborne [Andersen et al. 2002]. Also, an essential element of "niche based theories of species diversity" suggests that spatial heterogeneity in environmental conditions is a critical cause of species diversity. Therefore, a more heterogeneous area can provide more suitable conditions for a larger number of species [Chase & Leibold 2003; Hutchinson 1957].

With high occupancy environments and the risk of high humidity, air ventilation is critical in indoor environments. Windows may help to eliminate moisture build up on the surfaces of indoor material to decrease microbial growth [Jamriska et al. 2000]. Windows however can also provide an area of great microbial growth. With the opening of windows and changes of weather and precipitation, microbes can use window sills as substrates to grow. Thus frequent cleaning around windows in indoor environments is important to decrease microbial growth on this substrate.

Highly populated indoor environments can also lead to a higher production of indoor dust, which contain organic substrates that can act as a major source of nutrient that enable

microbes to grow [Brown et al. 1996]. Another factor that can affect dust and microbial growth in indoor environments is the use of household vacuums. Passive accumulation of particles is formed from the surrounding environments, and therefore indoor microbial assemblages can be shaped and even enriched by household vacuum cleaners or HVAC systems without a high efficiency particulate air (HEPA) filtration. Fungi and dust can build up in these dark spaces and act as an incubator and disperse fungal spores indoors [Cheong & Neumeister-Kemp 2005; Sharpe et al. 2014]. As a result, cleanliness of built environments is another factor that can correlate with fungal growth in indoor environments.

2.3 Fungal growth on construction and finishing materials

Fungi can grow on many different types of materials and substrates. Temperature, humidity, and type of surface are the three important factors that could determine fungal growth in indoor environments.

2.3.1 Wood

Damage to an indoor structure made of wood due to microorganisms can be an economically costly occurrence. In 1977 the UK estimated the cost of repairing fungal damaged timber used in construction amounted to £3 million per week [Schmidt 2007]. Wood is highly susceptible to fungal colonization by fungi such as *Cladosporium, Penicillium, and Aspergillus* [Bjurman 1994]. In addition, basidiomycetes such as white rot and brown rot fungi can also grow on these materials and lead to their decay, with *Serpula lacrymans* being the most common indoor basidiomycete [Schmidt 2007]. Drying wood leads to a higher nitrogen concentration and lower molecular carbohydrates on the surface, leading to a higher susceptibility to mould growth [Thelander et al. 1993, Viitanen 1997]. Some engineered wood products such as Oriented Strand Boards (OSB), plywood, and Medium Density Fiberboard (MDF) are more susceptible to growth of *Aspergillus, Trichoderma, and Penicillium* than solid wood and wood composites [Mankowski & Morrell 2000], while woods such as Douglas-fir heartwood provide more durability and are less susceptible to these microrganisms [Yan & Morrell 2015].

2.3.2 Wallpaper

Both paper and glue are good media for fungal growth and thus wallpaper is very susceptible to mould growth [Bissett 1987; Grant et al. 1989]. Synthetic polymers such as synthetic rubber and plastic can also be degraded by *Aspergillus niger, Aspergillus flavus, Aureobasidium pullulans, Chaetomium* sp., *Penicillium funiculosum, Penicillium luteum*, and *Trichoderma* sp. [Flannigan et al. 2001].

2.3.3 Plastics and glass

Fungi can also grow on polyethylene and polyvinyl chloride (PVC) as they can degrade plasticizers including organic acid esters [Webb et al. 2000]. Glass reinforced plastics (GRP), known as fiberglass, and fiberglass ceiling tiles are other susceptible materials that can support fungal growth, especially *A. versicolor*, and *Penicillium* sp. [Horak et al. 1985, Steyn & Vleggaar 1976].

2.3.4 Paint

Fungi can also grow on water-based or solvent-based paints; however, it isn't clear whether moulds found on the surface are using the paint components or taking nutrients from the dust on the surface [Allsopp et al. 2004]. In general, paints can increase or decrease the susceptibility of a given base material, depending on the type of fungi growing on the substrate. For example, paints can prevent the growth of *Aureobasidium pullulans*, while *Penicillium* and *Aspergillus* species can grow rapidly on paints [Nielsen 2003].

2.3.5 Carpet

The presence of carpets indoor can also influence fungal growth [Sharpe et al. 2014; Wnni et al. 2014]. High humidity that makes carpets wet, is responsible for producing a suitable growth condition for fungi such as *Alternaria* sp. [DEHS 2010; Wani et al. 2014]. There are many ways to prevent fungal contamination of carpets such as the use of surface disinfectants, removal of wet organic material regularly, and proper ventilation [Wani et al. 2014].

2.3.6 Dust

Dust is a mixture of organic and inorganic airborne air particles, originating from the indoor and outdoor environments [Scott et al. 2004]. The main constituents of house dust are plant pollen, human and animal hair, live and dead dust mites, textile fibers, paper fibers, human skin cells and other organic debris [Brown et al. 1996]. Exposure to house dust is mainly via inhalation, but to a lesser extent also by mouth, hand activities, and ingestion of dust [WHO 2009]. These organic substrates act as a major source of nutrients that enable organisms such as animals (arthropods, rodents), bacteria and fungi to grow on the surface of building materials such as steel, glass, brick, concrete, gypsum drywall, and stones [Brown et al. 1996; Harvey & May 1990; Scott 2001]. The main components of the dust microbiome are viable and non-viable fungal spores, fragments of spores, hyphae, lichen soredia, fruiting bodies, bacterial cells, endospores, and fragmented cells [Pitkaranta 2012]. Therefore, dust analysis may be used as a means to observe the presence of fungal species that have accumulated over time in a room [Portnoy et al. 2004]. This method has been particularly used in earlier monitoring studies [Abdalla 1988; De-Wei & Kendrick 1955; Wani et al. 2014].

2.3.7 Gypsum boards

Drywall (also known as plasterboard, wallboard, gypsum board, or LAGYP) is a panel made of gypsum plaster pressed between two thick sheets of paper. Drywall is a ubiquitous building material used in all construction and was invented in early 1900s, but did not become predominantly used until after War World II [Wakili et al. 2014]. It is used to make interior walls and ceilings of buildings and is sold under the trademarks Sheetrock, Gyproc, and Gyprock. North America is one of the largest gypsum board users in the world. These drywall pieces serve not only as a thermal store but as a heat exchanger as well [Wakili et al. 2014].

A manufactured wallboard panel consists of a layer of gypsum plaster sandwiched between two layers of paper [Mehta et al. 2008]. Gypsum is a mineral found in sedimentary rock formations in a crystalline form known as calcium sulfate dihydrate $CaSO_4 \bullet 2H_2O$. The raw gypsum, $CaSO_4 \cdot 2 H_2O$, is heated (350^oF) to drive off the water then slightly re-hydrated to produce the hemi-hydrate of calcium sulfate ($CaSO_4 \cdot ½ H_2O$) [Mehta et al. 2008; Chandara et al. 2009; Wakili et al. 2014]. These gypsum crystals also provide good compressive strength properties to the final composite/sandwiched material system [Chen et al. 2010]. The plaster is then mixed with fiber (typically paper and/or fibreglass), plasticizer, foaming agent, and ground gypsum crystal as an accelerator, EDTA, and other additives that may decrease mildew and increase fire resistance (fibreglass and vermiculite) [Wakili et al. 2014]. The board is then formed by sandwiching a core of the wet mixture between two sheets of heavy paper or fibreglass mats. The sheets of paper, which are made up of cellulose and starch [Pasanen et al. 1997], provide tensile strength to the board and hold the gypsum together to give them a smoother edge for easier application. When the core sets, it is then dried in a large drying chamber, and the sandwich becomes rigid and strong enough for use as a building material. The mechanical strength origin of these building materials is still unclear (Chen et al. 2010).

Advantages of using drywall include ease of installation, fire resistance, sound isolation, durability, versatility, and their installation at low costs. However, their major disadvantage is their cellulosic paper backing which is susceptible to microbial growth [**Figure 2.2**]. Drywall can be exposed to microorganisms during manufacturing, storage, transport, and the environment where it is installed. Neighbouring materials installed with a high moisture capacity could diffuse to drywall and lead to higher humidity [Dedesko & Siegel 2015]. Insulating material for energy efficiency can also create potential for dampness and condensation [Dedesko & Siegel 2015]. After fungal growth is evident in buildings, the material must be ripped out and replaced. The annual loss of drywall due to microbiological damage in the building industry remains a problem.

Figure 2.2 Fungal growth on indoor drywall material [Boni 2000].

Drywall can have many different categories with varying lengths and thicknesses to suit different applications [**Table 2.1**] [Hess et al. 2009]. Although Menetrez et al. (2007) concluded that variations in type of gypsum have little effect on growth, there have been many new innovative categories of drywall that are water resistant and lead to less fungal growth. In addition, the gypsum itself can support fungal growth due to its nutrient content and additives that make it more hygroscopic at lower humidity levels [Andersen et al. 2011]. Nevertheless, the understanding of indoor microbial composition on drywall materials, both quantitatively and qualitatively, is not yet extensive in the literature and more investigations are required.

Table 2.1 The different categories of drywall available and their description and utility [Home Depot, Home Hardware, RONA 2015]

Category of drywall	Description
Fire-resistant (Type X)	Different thickness and multiple layers of wallboard provide increased fire rating. Often perlite, vermiculite and boric acid are added to improve fire resistance.
Gypsum "Firecode C".	This board is similar in composition to Type X, except for more glass fibers and a form of vermiculite, used to reduce shrinkage.
Greenboard	Drywall that contains an oil-based additive/wax in the green colored paper covering that provides moisture resistance. It is more expensive. Its paper covering resists water (but is not water-proof); can be used in washrooms and other high humidity areas.
Blueboard	Is a plaster base board with blue coating to be specifically finished with veneer plaster. It has high water and mould resistance. Plastering requires a skilled professional and is more expensive than Greenboard.
Cement board	This is more water-resistant than Greenboard, for use in high humid areas.
Soundproof drywall	Is a laminated drywall made with gypsum and other materials such as damping polymers to significantly increase the sound transmission class rating.
HUMITEK: Mould resistant drywall (new)/DensArmor paperless drywall	Paperless drywall. Gypsum is covered with fiberglass.
Lead-lined drywall	Drywall used around radiological equipment.
EcoRock/Enviroboard	Drywall that uses a combination of 20 recycled materials and no starch cellulose; it is advertised as being environmentally friendly.
Controlled density (CD)	Also called ceiling board, which is available only in 1/2" thickness and is significantly stiffer than regular boards.
Phase change drywall (new)	Stores and releases heat to save power. 26-45% paraffin.

2.3.8 Biodeterioration and biodegradation

Fungi that live in buildings are saprotrophs and are key agents in structural decay [Scott et al. 2004]. Fungal growth can degrade material and affect the structural integrity of a building [Portnoy et al. 2004]. The degrading enzymes and metabolites produced by the microorganisms induce hydrolysis of cellulose macromolecules, leading to discoloration, mass loss, and an increase in damage of internal areas of the material [Webb et al. 2000; Pekhtasheva et al. 2012]**.** Most of the natural and man-made compounds, in particular construction and finishing materials that contain natural organic polymers including starch, cellulose, hemicellulose, pectin, and lignin are most susceptible to fungal growth [Block 1953; Flannigan et al. 2001]. Inorganic compounds can also serve as a medium for fungal growth with the support of the dust available in the atmosphere [Korpi et al. 1997; Pasanen et al. 1997]. The physical and mechanical properties of these indoor materials can be affected over time by fungal growth. Next to the microbially driven biodegradation mechanism, 'biodeterioration' of the material can also take place, by definition due to *abiotic* factors such as temperature and UV radiation, and lead to weight loss over time [Pekhtasheva et al. 2012, Ramirez et al. 2011].

Tensile testing using an Instron machine is one of the many methods used to determine the mechanical properties of a material [**Figure 2.3A**]. This test determines the behaviour of a material with forces being applied in tension [Instron]. A sample is pulled apart to find its strength and elongation capabilities. Tensile stress and strain are calculated and plotted as a stress-strain diagram [**Figure 2.3B**], which can determine the Young's Modulus of elasticity and the ultimate stress (UTS) or maximum stress a material can withstand before the break or rupture point.

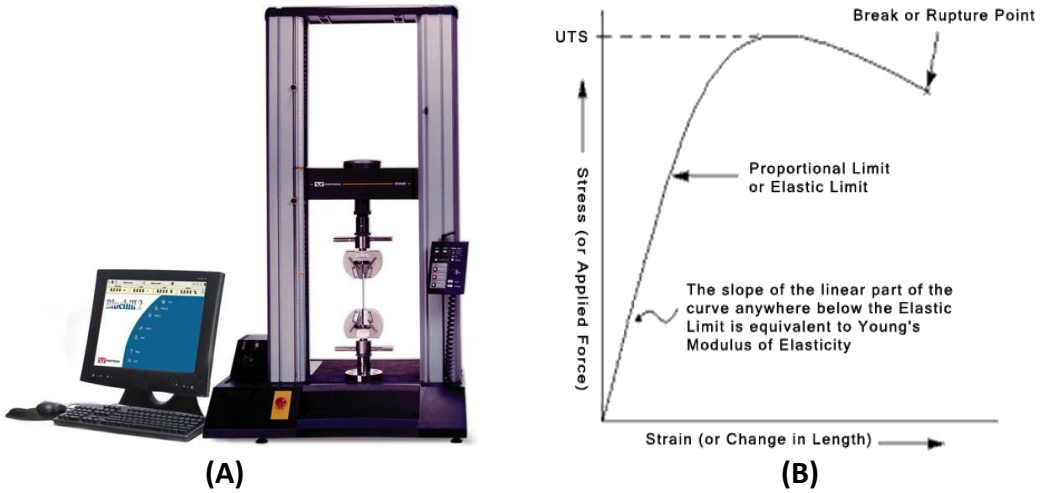
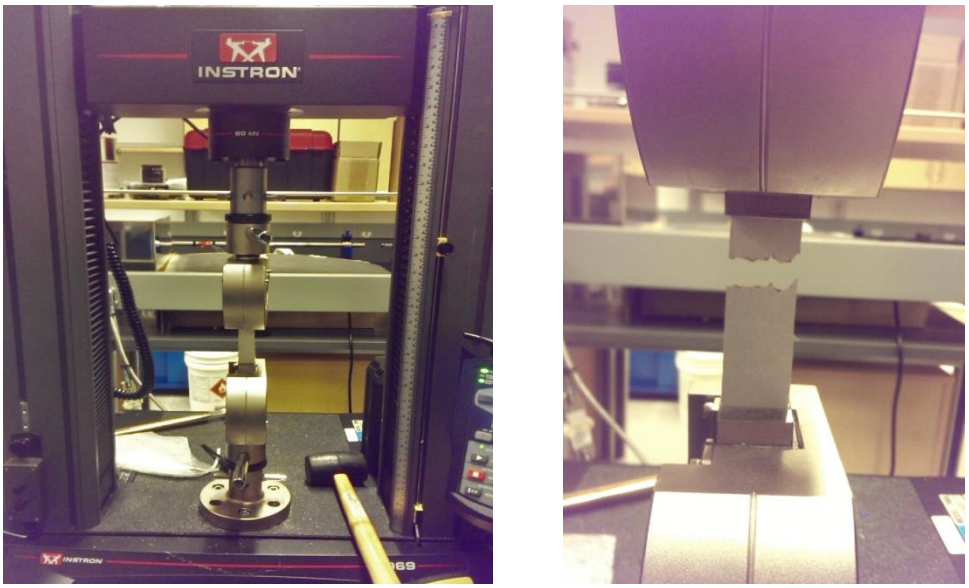


Figure 2.3 (A) Instron machine used for tensile testing, and (B) the stress versus strain graph of a specimen after a tensile test [Instron].

When conducting a tensile test, the specimen shape and dimensions, and the choice of grips are critical [**Figure 2.4A**]. The shape is usually chosen from a standard of the material and it is important to avoid breaks and or fractures at the site of grips [**Figure 2.4B**]. Grip type selection is also another important factor to ensure no slippage or breakage in the gripping area. Once the shape and grips for the specimen have been chosen, a vertical alignment is performed to avoid side bending of the material.



(A) **(B)**

Figure 2.4 (A) Close-up configuration of a test sample under tensile loading, using Wedge Action Grips for flexible thin specimens, and (B) induced fracture in the middle of the specimen, without slippage at grip sites.

2.4 Fungal products: Mycotoxins

Fungi produce a large number of secondary metabolites, including plant growth regulators (e.g., gibberellins), pharmaceutically-valuable compounds such as antibiotics (e.g., penicillin), and immunosuppressants (cyclosporin), pigments (carotenoids), and toxins [Keller et al. 2005]. Different toxic metabolites produced by fungi, based on their concentration and targets, are categorized into: i) products that are mainly toxic to bacteria known as antibiotics, ii) fungal metabolites known as phytotoxins that are toxic to plants, iii) mycotoxins that are by-products of microfungi, mostly non-volatile, low molecular weight compounds below 1500 Da, which cause a toxic response at low doses, iv) other low molecular weight metabolites that are toxic at high concentrations such as ethanol (vapour-phase chemicals) [Bennett & Klich 2003; Nielsen 2003]. Particulate chemicals with 10 µm aerodynamic diameter or below are respirable and can be deposited in the lower alveoli. In contrast, airborne particulates with <0.1 µm behave like

vapour based chemicals, and can move in and out of the airways with respiratory movements [McGregor 1987].

It has been 54 years since mycotoxins were discovered [Bennett & Klich 2003]. Mycotoxins have caused epidemics such as: i) ergotism from mouldy bread and alkaloids of *Claviceps purpurea* that led to the death of hundreds of thousands of people, ii) alimentary toxic aleukia (ATA) that killed 100,000 Russians during 1942-1948, iii) stachybotrytoxicosis that killed many horses in the USSR in the 1930s, and iv) aflatoxicosis which killed more than 100,000 turkey spoults in the United Kingdom in 1960 due to mould-contaminated peanut meals [Bennett & Klich 2003; Pitt 2000]. Many different fungal species are capable of producing mycotoxins that are toxic to humans. Based on section 2.2 and the findings in this section, five of the most problematic and common mycotoxin-producing fungi found in indoor environments have been summarized below [**Table 2.2**].

Table 2.2 Fungal mycotoxin production, their growth, and health effects.

Fungi	Mycotoxins	Location	Effects	References
Aspergillus versicolor	Sterigmatocystin cyclopiaxonic acid	On nutrient poor and low a_w: dust, concrete, plaster, and wallpaper. Commonly found in soil, hay, cotton and dairy products.	Most toxic, Class 2B carcinogenic, and mutagenic, but lower than aflotoxin. Can affect the liver and kidney and cause diarrhea and upset stomach.	[Brown et al. 1996; DEHS 2010; Nielsen 2003]
Aspergillus fumigatus	Gliotoxin Tripacidin	Commonly found outdoors in compost piles with temperatures > 40^0 C, in soils, and on cereals.	Apoptosis inducer. Decreases immune function, affects CNS function, can cause aspergillosis, and is mutagenic.	[DEHS 2010]
Aspergillus niger	Fumonisins (B_2, B_4, and B_6) ochratoxins	Most dominant indoor airborne fungal species found on textiles, in soils, grains, fruits and vegetables.	Carcinogenic, skin and pulmonary infections, and fungal related ear infections.	[Ababutain 2013; DEHS 2010; Frisvad et al. 2011]
Alternaria alternata	Tenuazonic acid and other toxic metabolites	Commonly found on carpets, textiles, horizontal surfaces, window frames.	Common allergen, may be related to bakers asthma, bronchiospasms, and pulmonary emphysema	[DEHS 2010; Sharpe et al. 2014]
Stachybotrys chartarum	Trichothecenes	The most significant indoor fungi on water damaged material: (gypsum drywall, wallpaper, insulation) and other cellulose based material (ceiling tiles).	Can inhibit DNA/RNA synthesis, cell division. If ingested by animals: necrotic lesions, lower platelet/WBC, nervous system disorders, and death. If ingested by humans: fatigue, neurological disorder, impair lung, and sick building syndrome.	[Andersen et al. 2002; Bin-Umer et al. 2011; Croft et al. 1986; Etzel et al. 1998; Forgacs & Carll 1962]

Some species of fungi are capable of producing a variety of mycotoxins. Production may also vary depending on the interaction with other fungal species and other microbes, water activity, temperature, gas composition and the presence of chemical preservatives [Bennett & Klich 2003; Varga et al. 2005]. It is theorized that mycotoxin production occurs in idiophase when fungal growth is halted, usually because of the nutrient exhaustion [Demain 1986]; however, there is also evidence of mycotoxin production during active growth of fungi [Magan & Aldred 2007]. Other studies show that mycotoxin production was stimulated by the initiation of stress, particularly drought stress, when conditions were marginal for growth [Magan & Aldred 2007]. For instance, *Aspergillus carbonarious g*rows optimally at 30-35^{0}C and 0.95 a_w, but instead of growth *A. carbonarious* produces ochratoxin A (OTA), which is a mycotoxin associated with nephrotoxicity, hepatotoxicity, immunotoxicity effects on animals or the human body [Esteban et al. 2004; Hohler 1998]. In the following sub-sections two fungal mycotoxins including Sterigmatocystin and Trichothecenes will be reviewed, due to their significance in indoor environments.

2.4.1 Sterigmatocystin

Sterigmatocystin is an intermediate product in the aflatoxin biosynthetic pathway or the end-product in different ascomycetes. It is produced by 20 different species of fungi such as *A. versicolor* and *A. nidulans* [Brown et al. 1996]. *A. versicolor* is one of the most frequently occurring species identified in damp indoor environments, as a result of its ability to grow on very nutrient poor materials and low a_w such as dust, concrete, and plaster [Nielsen 2003]. Also more was found in non-sporulating mycelium in contrast to conidia [Nielsen et al. 1998]. Sterigmatocystin is among the most toxic, mutagenic, and carcinogenic natural products, but considerably lower than aflatoxins [Brown et al. 1996]. This toxin has major effects on the liver and kidney, and is classified as a class 2B carcinogen [Brown et al. 1996].

2.4.2 Trichothecenes

Trichothecene mycotoxins are produced by different genera including *Fusarium, Spicellum, Stachybotrys, Cephalosporium, Trichoderma,* and *Trichothecium.* Fungi with trichothecene production abilities are responsible for the spoilage of cereal crops and various fruits; however, among these genera, *Stachybotrys* has been demonstrated as a significant indoor environment contaminant which is associated with damp building-related illnesses [Nielsen 2003; Yike et al. 2007]. In general, trichothecene-producing species can grow on water damaged building materials (e.g., gypsum boards, wallpaper, and insulation) and any cellulose-based building materials [Andersen et al. 2002].

These active compounds are capable of inhibiting eukaryotic protein synthesis, inhibiting DNA, RNA synthesis, cell division, membrane structure, and mitochondrial protein synthesis [Bin-

Umer et al. 2011]. Ingestion of this mycotoxin has been associated with necrotic lesions, lowered platelet and WBC count, nervous system disorders and death [Forgacs & Carll 1962]. It may also cause chronic fatigue, neurological disorders, impaired lung functions and sick building syndrome [Croft et al. 1986; Etzel et al. 1998]. Residents of houses contaminated with trichothecenes may suffer from illnesses such as the cold, flu, sore throat, diarrhea, headaches, fatigue, dermatitis, and alopecia [Croft et al. 1986].

2.5 The indoor microbiome and health problems

The potential health problems may be categorized into three groups: i) general symptoms including fatigue, memory and concentration problems, nausea, and a poor immune system, ii) mucosal symptoms such as blocked nose, itching eyes, burning skin sensation, and recurrent upper airways infections, and finally iii) lung symptoms such as wheezing, cough, bronchitis and asthma [Bin-Umer et al. 2011].

Smaller-sized fragments have longer lifetimes in the air compared to larger spores, and can penetrate into the alveolar region when inhaled. Moreover, due to their small size, fragments may be able to evade phagocytosis by macrophages and can be transported through systemic circulation [Cabral 2010]. The health impact of these fragments may therefore be greater than that of spores [Cabral 2010].

Fungi and their products are associated with poor indoor air quality and sick building syndrome [Karunasena et al. 2000]. Sick building syndrome (SBS) is a term commonly used to describe the consequence of poor indoor air quality [Karunasena et al. 2000]. This term, coined in 1982, was used to describe the many symptoms that are commonly observed [Karunasena et al. 2000].

Numerous studies have demonstrated that people living and working in damp or mouldy buildings have an increased risk of airway infections, respiratory diseases such as mycoses, hypersensitivity pneumonitis (HP), organic dust toxic syndrome (ODTS), as well as cancer [Cabral 2010; Douwes et al. 2003; Mihinova & Pieckova 2012]. It has been estimated that 21% of US asthma cases are attributed to dampness and mould exposure [Mihinova & Pieckova 2012]. In Sweden, Wickman et al. 1992 found that *Penicillium*, *Alternaria*, and *Cladosporium* moulds were more common in homes of children with allergies. Asthma is a serious chronic inflammatory problem that can be generated by a variety of allergens, such as pollen, animal dander, dust mites, air pollutants, and moulds [Brunekreef et al. 1989]. The risk of opportunistic infections is also on the rise [Brunekreef et al. 1989].

Health problems associated with mycotoxins depend on their type, duration and dose of exposure, age, health and nutritional status of the affected individual [Adan & Samson 2011]. Acute toxicity of mycotoxins can lead to degeneration of liver or kidney or interfere with protein synthesis. Chronic toxicity may result in cancer or tumor induction [Adan & Samson

2011]. Some may even interfere with DNA replication and produce mutagenic or teratogenic responses [Adan & Samson 2011; Varga et al. 2005]. Although the key role of microbial growth and emissions in building-related illnesses have been hypothesized, the evidence for its proof is still weak [Pitkaranta 2012].

2.6 Sampling methods

Fungi in indoor environments can be sampled from the air, surfaces (eg. tape lifting), dust, and materials. Sample detection techniques that require a short time, have reduced labour, and great analytical frequency are still needed. When air sampling, special features need to be considered. Differences in aerodynamic diameters of particles can affect collection. Short sampling times are also a common problem while air sampling. One method of air sampling is by collecting bioaerosols in microcentrifuge tubes [Lindsley et al. 2006], which can later be analyzed by PCR and immunoassays.

It has been found that dust microbial measurements can reflect microbial load present over a period of time longer than can be obtained using air samples [Portnoy et al 2004]. Thus by collecting dust samples, evaluation of long term exposure is possible. It has been noted, however, that single sample dust collections are not sufficient to provide satisfactory concentrations of fungi present in indoor environments. There are spatial and temporal variations in microbial concentrations and thus more than one sample would provide a better representative of fungal communities present in the air. Outdoor samples should also be taken for comparison, since it is an important source for indoor air [Mazur & Kim 2006].

Using these different methods of sampling requires further analysis to determine the microbial communities present in indoor environments. One of these methods is the use of traditional culture-based techniques. Many of these techniques, however, have limitations that result in imprecise and inaccurate findings of indoor compositions both quantitatively and qualitatively [Lee et al. 2010]. The key problem relates to selectivity and low resolution of such methods. For example, traditional plate cultivation may only detect certain viable organisms and produce results in laboratory conditions, but not those that are unculturable, dormant, or dead [Pitkaranta 2012]. Some species also grow more rapidly than others and could over run others on the culture plate, suggesting that many species may be missed if relying on culturing alone. Thus a comprehensive study to characterize the main constituents of the built environment microbiome using advanced DNA-based methods may offer solutions to these problems [Mendell et al. 2011]. DNA-based methods are inexpensive, rapid and an accurate and standard method that can be used for fungal DNA amplification.

2.7 Summary

Drywall is a common material used in construction of houses. Microbial growth, such as by fungi, is a frequent problem and disadvantage of this type of material. In the past, research on factors affecting indoor fungal growth on the gypsum drywall material has been limited. For this reason, an understanding of the diversity of microbial communities such as fungi on this highly common building material was deemed essential. Moreover, there still exists a gap in the literature on understanding the effect of grown microbial communities on the physical and mechanical properties of building materials. Thus an inter-disciplinary study is required and can provide additional information on this topic. Such comprehension can eventually lead to better design of new buildings, optimum selection of related materials, and reduction in human health risks in built environments.

Chapter 3. Materials and Methods

3.1 Sampling

Sampling for this study was conducted at the University of British Columbia's Okanagan campus, Kelowna, Canada (July 2014). In order to determine which building attributes or environmental conditions of built environments may influence the fungal growth on drywall, three main environmental factors were chosen: i) age of building, ii) different function/types of rooms, and iii) type of flooring [**Figure 3.1**]. A nested case control design was used for this study. Building assessments and some random effects (i.e., other uncontrolled factors which were observed but could largely vary from room to room) such as temperature, humidity, occupancy level, window presence, and general cleanliness were also monitored in each room [**Appendix A**]. Indoor temperature and humidity were measured by an indoor Solar Powered Wireless Humidity/Temperature Station and Sensor (model WS-6020U-IT) in the morning, afternoon, and evening hours during the month of July 2014 to obtain the average temperature, as well as the relative humidity of each environment. Occupancy level was defined as low (<10), medium (10-20), and high (>20), while general dustiness was defined as 1-very clean, 2-moderate, and 3-unclean.

Regarding the first main factor, to examine the difference in fungal growth between constructed buildings of different ages, samples were taken from four buildings: i) newly constructed buildings on campus: Arts and Sciences Centre (ASC), and Engineering/Management/ Education (EME), and ii) older constructed buildings on campus: Arts (ARTS), and Science (SCI) [**Table 3.1**]. It's important to note that the expansion of the Science and Arts buildings took place in 2005 and led to the construction of the third floor in both of these buildings. To compare different functions and types of rooms, three different types of rooms were chosen for each building: i) offices, ii) classrooms, and iii) laboratories. The type of flooring for these rooms was also compared. Offices with and without carpets, classrooms with and without carpets, and laboratories without carpets were compared in all four buildings (there were no laboratories with carpet). A total of 51 rooms across campus were sampled [**Appendix B**].

Table 3.1 Details of the four buildings sampled at UBCO campus.

	Primary Use	Construction Type	# of Floors	Presence of Atrium	Year Built
ASC	Research	Concrete	4	No	2010
EME	Research	Concrete	5	No	2011
ARTS	Classroom	Steel frame	3	Yes (enclosed)	1992
SCI	Research	Steel frame	3	Yes (open)	1992

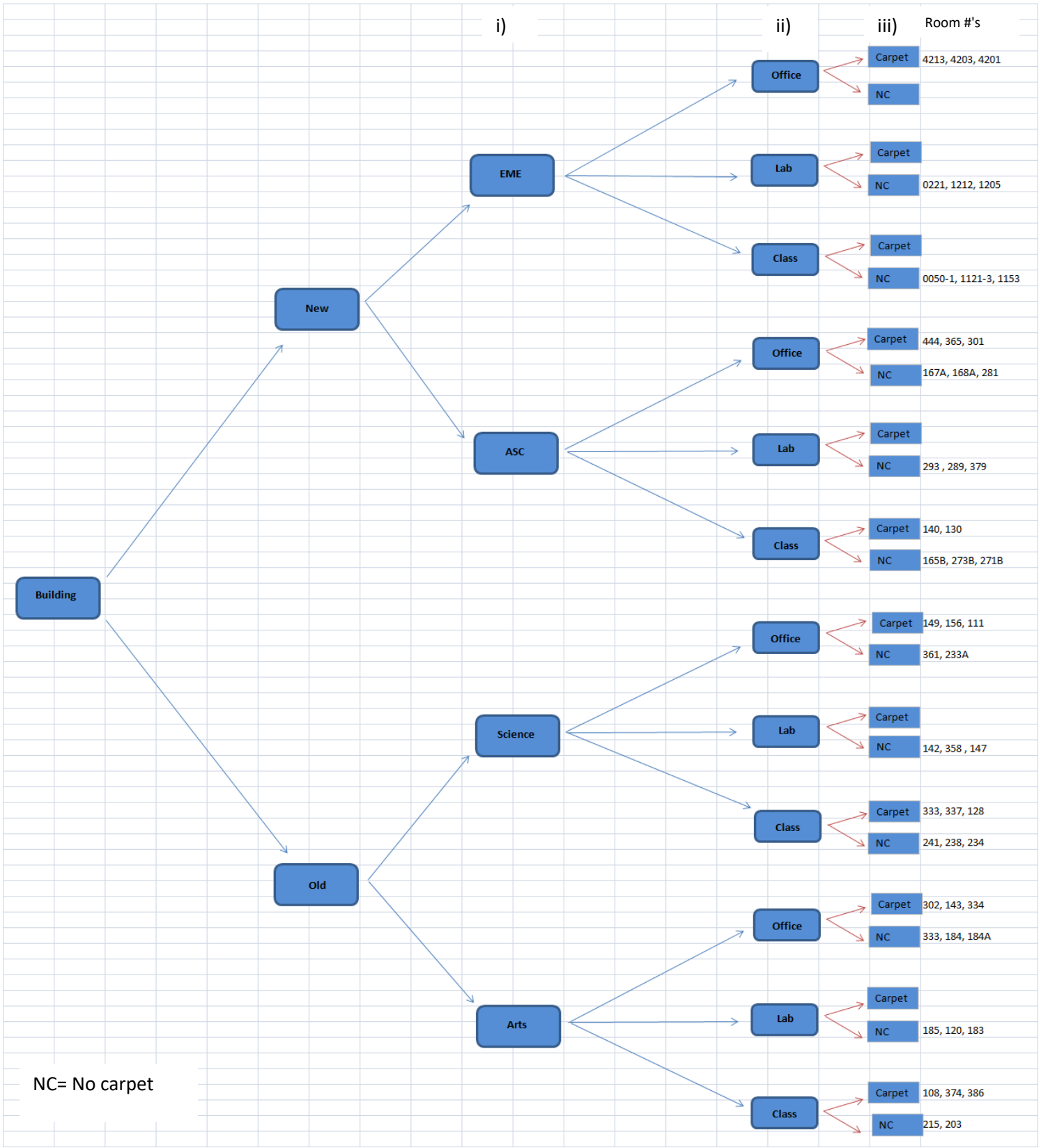


Figure 3.1 51 selected rooms for identified environmental control factors at UBCO: i) age of building, ii) type/function of room, and iii) type of flooring.

Samples of airborne microfungi were collected using a Petri plate gravitational dust settling method [Wakili et al. 2014]. Dust samples from the 51 rooms were collected over a one-month time period in July 2014. Two Petri dishes per room (102 Petri dishes) were set up 2.5 m above ground level in each selected environment. Each of the Petri dishes was placed on separate shelf brackets [**Figure 3.2A**] and attached to the wall with tape (102 shelf brackets). After the dust samples were obtained, the Petri dish lid was closed. In a sterilized environment (flow hood), the dust was extracted from each Petri dish using sterilized aqueous solution with 0.5% Tween 20 (Amresco, Solon, OH, USA). This solution was added to each Petri dish, swirled, and the contents were poured into a separate sterilized clear vertical graduated glass container and capped (Fisher Scientific, Ottawa, Ontario, Canada) [**Figure 3.2B**]. This process was repeated until there was 125ml of water at the bottom of each container. This water was added in order to maintain 95-99% RH within the container for optimum growth.

(A) **(B)**

Figure 3.2 (A) Petri dishes setup on shelf brackets for dust collection, and (B) 500 ml graduated volumetric bottles with rubber lined caps.

Gypsum boards were considered as a representative building material in the study. Namely, regular drywall (4 ft X 8 ft X 1/2" CGC Sheetrock® Brand Ultralight Panels) was purchased from a nationwide hardware retail store (Home Depot) and cut into pieces of 5cm x 8cm. The drywall pieces were sterilized under ultraviolet radiation for 24 hours and turned to expose all edges (rather than autoclaving, as this would likely affect the physical properties of the material). Fifty-one pieces of drywall were pre-weighed for physical testing. All 102 sterilized drywall pieces were placed vertically in each container containing the dust collected plus water, the bottom section submerged in water, and the lid closed. The drywall samples were stored at 25^0C for 4 weeks.

3.2 Growth coverage

After a 4-week growth phase, one sample from each room was tested for % growth coverage on the drywall sample using Image-J software [Sun et al. 2015]. A scale of 1-5 was used to categorize the % growth, as defined below:

1 = minimal growth, (growth covering 0-20% of sample area)

2 = growth covering 20-40% of sample area

3 = growth covering 40-60% of sample area

4 = growth covering 60-80% of sample area

5 = growth covering majority (80-100%) of sample area

3.3 Fungal growth identification

After determining the % coverage of the drywall samples, they were assessed for fungal taxa that are able to grow on gypsum boards in order to determine fungal diversity. The fungal diversity index was the number of genera of fungi found on each of the 51 drywall samples. Fungi growing on the drywall were transferred onto Potato Dextrose Agar (Difco, Detroit, Michigan, USA) and incubated at room temperature for one week. Pure cultures from all morphologically different colonies were grown and sub-cultured for identification to the species level when possible, using macroscopic and microscopic characteristics. Wet mount slides were made of the fungi using glycerol, and later examined for identification using a compound microscope.

3.4 PCR of fungal species problematic for health

After fungal identification by culturing methods, further testing was required. In particular, in order to better detect the presence and growth of fungal species that are known to be human pathogens, a DNA-based approach (here PCR amplification) was used to further determine the identity of the fungi present on the drywall samples. The drywall samples were examined for the presence of three pathogenic fungi that commonly grow on indoor wet materials, including *Alternaria alternata, Aspergillus versicolor,* and *Aspergillus fumigatus* [Brown et al. 1996; Nielsen 2003, Mihinova & Pieckova 2012].

3.4.1 DNA extraction

DNA was extracted from the drywall samples using the FastDNA® SPIN Kit for Soil (MP, Biomedicals, LLC, Solon, OH, USA) according to the manufacturer's instructions. Total DNA was extracted from 500 mg of fungi growing on 51 drywall samples. Extracted DNA samples were

checked for integrity by agarose gel electrophoresis. The purity of the extracted DNA was measured using NanoVue Plus™ spectrophotometer (GE Healthcare, Buckinghamshire, UK) before preserving at -20^{0}C.

3.4.2 PCR amplification

After DNA extraction, each sample was selected for PCR amplification. Three sets of indoor taxa-specific primers (IDT-Coralville, IA, USA) were selected for further identification of the fungi on drywall samples [**Table 3.2**]. PCR amplifications were carried out using a C1000 Touch™ Thermal Cycler (BioRad, Ontario, Canada). Each PCR mixture (50 µl) contained 10 µl of 5X Green Go Taq® Flexi Buffer, 200 µM dNTPs, 2 µl $MgCl_2$ (25 mM), 0.2 µM of each primer, 1.25 U of Go Taq® G2 Hot Start Polymerase (Promega, Madison, WI, USA), nuclease-free water (IDT-Coralville, IA, USA), and 2 µl of extracted DNA (5 ng/µl).

The PCR conditions started with an initial DNA denaturation (94^{o}C for 2 min), followed by 25 cycles of 1 min at 94^{o}C (denaturing), 1 min at 52^{o}C (annealing), and 1 min at 72^{o}C (extension), followed by a final extension of 5 min at 72^{o}C. Once the DNA was amplified, the procedure was analyzed to check for integrity by agarose gel electrophoresis. 10 µl of PCR product was mixed with loading buffer and loaded onto 2% agarose gel to control for PCR products. 5 µl of 1 KB ladder (Invitrogen) was also used to visualize the size of DNA fragments generated. A negative control without template was also used to test for PCR functioning.

Table 3.2 PCR primers used to identify the health hazardous indoor fungi on drywall specimens.

	Primer name (direction)	**Primer sequence (5' ⟶ 3')**	**Reference**
Primer set 1	*Aspergillus fumigatus* (forward)	GCCCGCCGTTTCGAC	[Walsh et al. 2011]
	(reverse)	CCGTTGTTGAAAGTTTTAACTGATTAC	
Primer set 2	*Aspergillus versicolor* (forward)	CGGCGGGGAGCCCT	[Blachere et al. 2007]
	(reverse)	CCATTGTTGAAAGTTTTGACTGATCTTA	
Primer set 3	*Alternaria alternata* (forward)	GGCGGGCTGGAACCTC	[Haugland et al. 2002]
	(reverse)	GCAATTACAAAAGGTTTATGTTTGTCGTA	

3.5 Physical and mechanical testing

Pre-weighed drywall samples were weighed again (i.e., after the fungal growth and drying the samples) to determine their final weight. Samples with different ranges of % growth coverage were randomly selected (n=25), along with controls (n=5), as well as samples that were exposed to high moisture but contained no fungal growth in order to test the pure effect of water absorption (n=5), for a total of 35 experimental units. The samples were placed in an oven at 65°C for one week to measure the dry weights **[Eqn 1]**.

Percent change in weight= [(Final weight– Initial weight)/Initial] x 100 **[Eqn 1]**

Similarly, drywall pieces were used for mechanical testing. Samples with varying ranges of % growth coverages were randomly selected (n=21), along with controls (n=5), and samples that were exposed to high moisture but contained no fungal growth in order to test the effect of water (n=5) for a total of 31 experimental units. For mechanical testing, the drywall samples were air dried and the paper backing separated from the gypsum. The 5cm X 8cm specimen pieces were then cut in half vertically. Tensile testing (via an Instron machine) was used to test whether the fungal growth had degraded the drywall material's strength over time. A modified ASTM D828-97, which is a standard test method for tensile properties of paper and paperboard using constant rate of elongation was used. The extension rate was set at 0.1 mm/min, with a load of 5N.

Microstructural characterization of the select drywall papers post mechanical testing was also conducted using a scanning electron microscope (SEM) on fractured surfaces. The SEM images were also examined to reveal the fungal growth on the drywall samples compared to the control samples.

3.6 Statistical analysis

The Statistical Package SPSS version 22 was employed for statistical analysis and data visualization. The variables in data sets were tested for normality and homogeneity of variance [**Appendix C**]. For each type of analysis where the normality was not met, an applicable non-parametric test was used. When the assumptions of normality were met, statistical significances (p-values) of samples were obtained using the standard ANOVA and related post hoc tests [**Table 3.3**].

Table 3.3 Summary of statistical methods used for different responses/analyses.

Measured Response/Analysis	Method
% Coverage (ordinal variable)	• Kruskal-Wallis Analysis Dunn-Bonferroni post hoc • GzLM
Fungal Diversity (ordinal variable)	• Kruskal-Wallis Analysis Dunn-Bonferroni post hoc • Chi-Square Test • GzLM
Correlation Between Coverage & Diversity	• Spearman's Rank Correlation Analysis
Dry Weight Loss (continuous variable)	• ANOVA Tamhane's post hoc
Tensile Strength (continuous variable)	• ANOVA Tamhane's post hoc
Correlation Between Weight Loss & Tensile Strength	• Pearson Correlation Analysis

Data analysis using the Generalized Linear Model (GzLM) was also performed for the coverage and diversity responses. GzLM is an extension of the general linear model (GLM), which allows regression analysis of non-normally distributed data. Since the dependent variables for % coverage and diversity were ordinal, the ordinal logistic model (link function) was chosen in GzLM. This multinomial logistic model relies on multiple regression equation [**Eqn 2**] given as:

$$Y=B_o + B_{(Age)} + B_{(Type)} + B_{(Flooring)} +E \qquad \textbf{[Eqn 2]}$$

Where B_i is a linear combination of unknown parameters to be fitted to data; E is the random error. No post-hoc is needed when using GzLM.

Since no building and rooms within sampled buildings were physically identical, combining the effects from rooms could be potentially biased. As a result, next to the standard GzLM, a nested GzLM model was also developed and analyzed [**Appendix D**]. Nested terms under this design were particularly useful for assessing the effect of a factor in a particular nest/building type without interacting with the levels of factors from other nests [IBM Knowledge Centre, 2015].

Chapter 4. Results and Discussion

4.1 Fungal coverage on drywall

When considering the tested indoor factors influencing the % coverage of fungal growth on drywall samples, the age of buildings ($\chi^2(1, N=51)=24.77$, $p=0.000$), and type of room ($\chi^2(2, N=51)=8.82$, $p=0.012$) were determining factors, while type of flooring had no significant effect ($\chi^2(1, N=51)=0.27$, $p=0.601$) [**Figure 4.1**]. As mentioned previously, although the third floor of the older buildings of Science and Arts were added later, no significant differences were observed for the rooms on the third floor in these buildings versus the first and second floor when observing fungal coverage, ($\chi^2(2, N=14)=2.34$, $p=0.311$) and ($\chi^2(2, N=14)=1.64$, $p=0.440$) respectively. Therefore, all of the rooms observed in these buildings were analyzed under the older building category. Older buildings led to a higher coverage degree of fungal growth on the samples as 3.7±1.1 (mean ± SD), while the newer built buildings led to a lower amount of fungal growth as 1.7±1.1 [**Figure 4.1A**]. The laboratories across the campus had a higher fungal coverage (3.7±1.2) when compared to offices (2.1±1.2) [**Figure 4.1B**]. No significant differences however were observed for the classrooms (3±1.6) compared to the laboratories and offices [**Figure 4.1B**]. Although the type of flooring did not have a significant effect using the Kruskal-Wallis test, carpeting (3±1.6) did show a favorable trend towards higher fungal coverage compared to no carpeting cases (2.7±1.4) [**Figure 4.1C**].

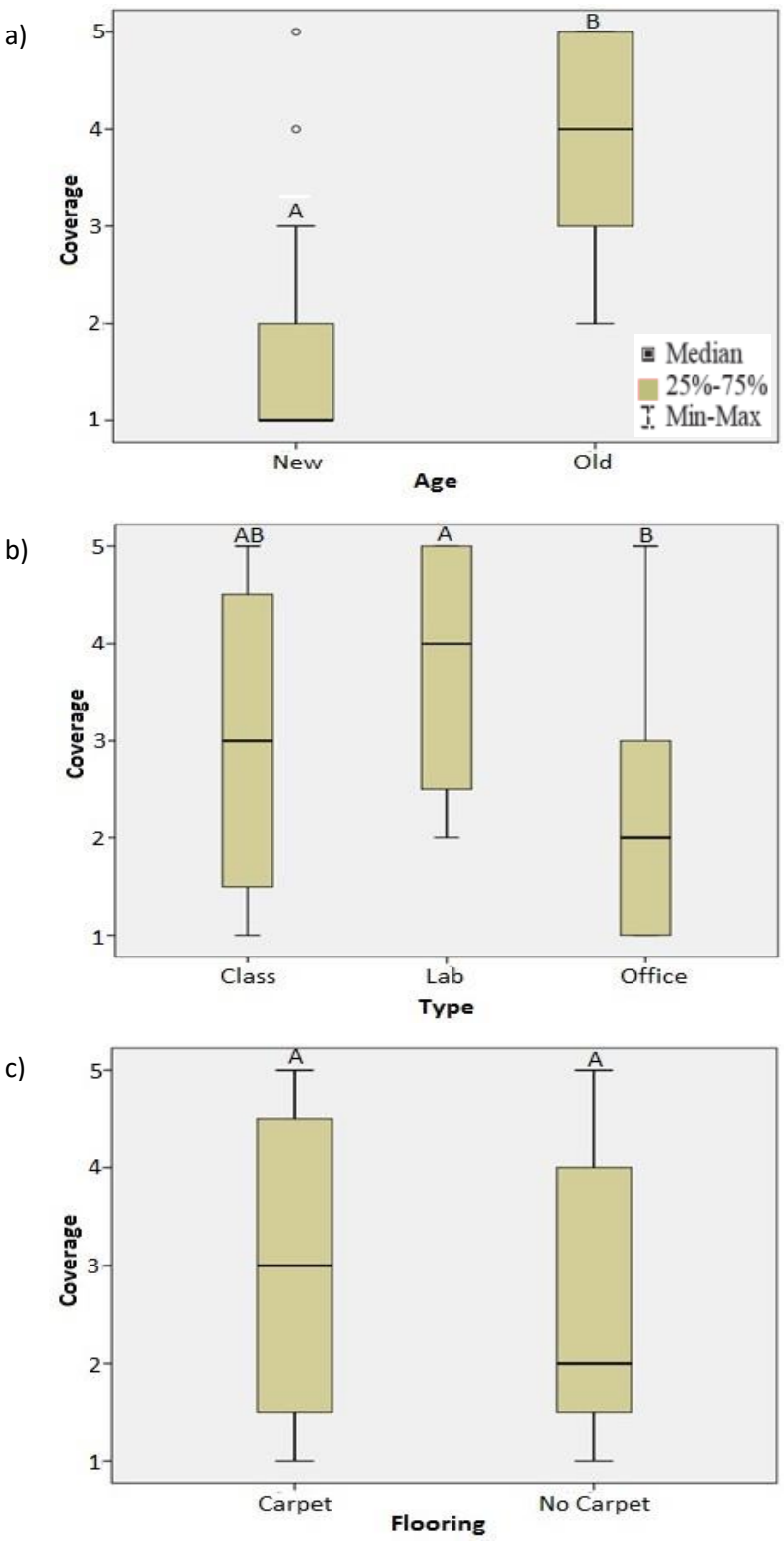


Figure 4.1 The relationship between indoor factors of (a) age, (b) type of room, and (c) type of flooring, influencing fungal coverage range (1-5) on drywall samples across 51 sampled rooms at UBCO. Different capital letters above boxplots indicate significant differences in % coverage between the factors using Kruskal-Wallis and Dunn-Bonferroni post hoc tests ($p<0.05$).

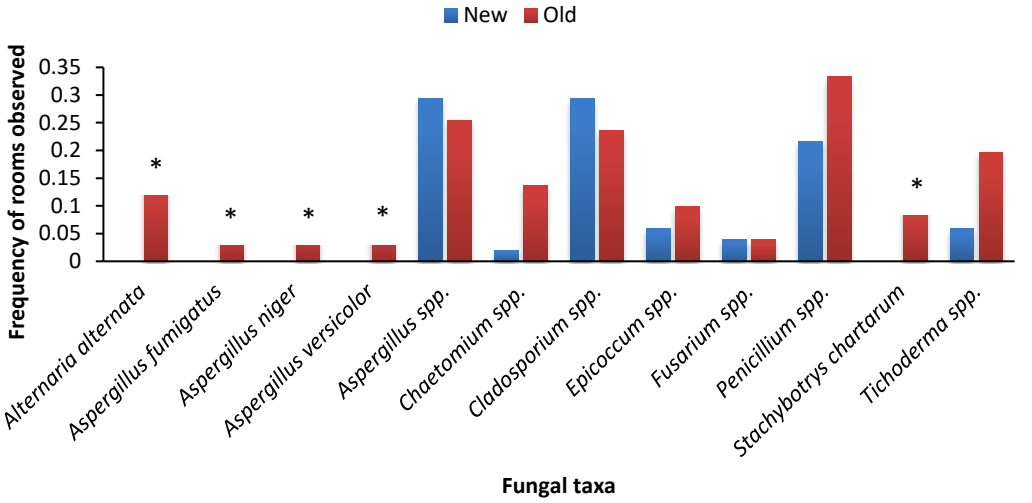
4.2 Fungal diversity on drywall

Morphological identification based on culturing and microscopy and the subsequent DNA extractions and PCR amplifications of drywall samples from 51 rooms, showed a diverse fungal community composition of 9 fungal taxa on the drywall; with *Aspergillus* spp*., Penicillium* spp*.,* and *Cladosporium* spp. being the most common, *and Alternaria* spp*., Stachybotrys* spp., and *Fusarium* spp. being the least common fungi found in indoor environments [**Table 4.1**]. When comparing the fungi present in rooms of older and newer buildings, there was a significantly higher proportion of rooms in older buildings with *Alternaria alternata* ($\chi^2(1)=7.00$, p=0.008), *Aspergillus fumigatus* ($\chi^2(1)=4.00$, p=0.046), *Aspergillus niger* ($\chi^2(1)=4.00$, p=0.046), *Aspergillus versicolor* ($\chi^2(1)=4.00$, p=0.046), and *Stachybotrys chartarum* ($\chi^2(1)=5.00$, p=0.025) [**Figure 4.2A**]. These five indoor fungal species hazardous to health were only found in older buildings [**Figure 4.2A**]. No significant differences in diversity of fungal genera were observed for the different types of rooms and flooring [**Figure 4.2 B** and **C**]. *Aspergillus niger* and *Stachybotrys chartarum* were detected using culturing and microscopy techniques, while *Aspergillus fumigatus*, *A. versicolor*, and *Alternaria alternata* were detected using PCR.

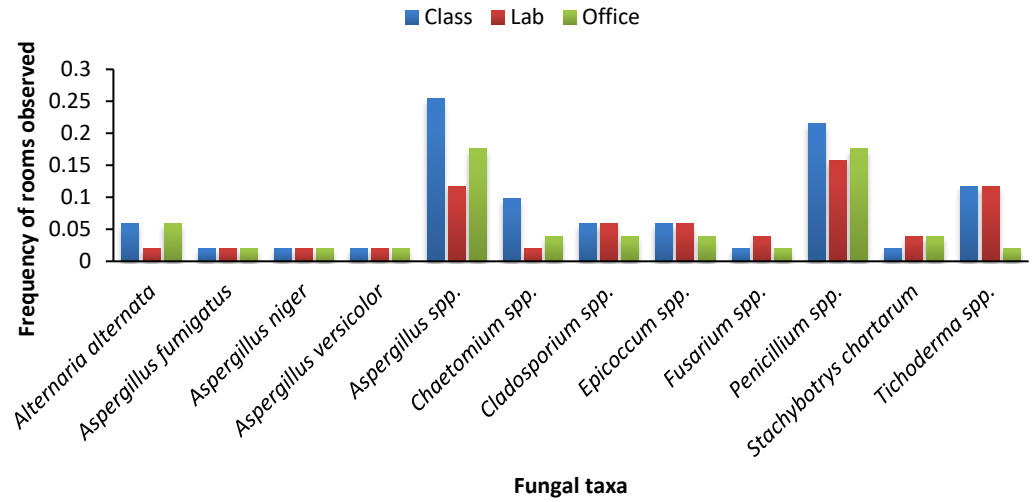
Table 4.1 Fungal community composition observed on the drywall samples of 51 sampled rooms.

Fungal genus	# of rooms observed	Proportion of rooms
Aspergillus spp.	37	0.725
Penicillium spp.	28	0.549
Cladosporium spp.	26	0.510
Trichoderma spp.	13	0.255
Epicoccum spp.	8	0.157
Chaetomium spp.	7	0.137
Alternaria spp.	7	0.137
Stachybotrys spp.	5	0.098
Fusarium spp.	4	0.078

A)



B)



C)

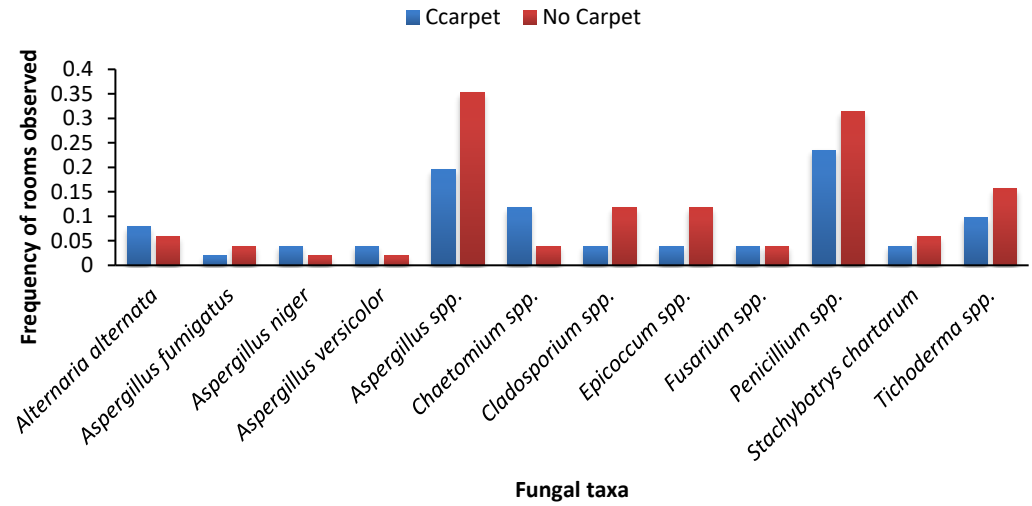


Figure 4.2 The fungal taxa present in rooms of buildings sampled at UBCO for the controlled factors of (a) age, (b) type of room, and (c) type of flooring. The single asterisk (*) indicates significant differences in frequency of rooms observed for each genus under the controlled factors, using chi-square test ($p<0.05$).

The fungal taxonomic richness (# of genera) was positively correlated with % coverage of fungal growth on the drywall ($R_s(51)=0.74$, $p<0.01$) [**Appendix E**]. The age of building ($\chi^2(1, N=51)=8.52$, $p=0.004$) and type of room ($\chi^2(2, N=51)=7.50$, $p=0.024$) were found to be associated with taxonomic richness, while type of flooring did not seem to be associated with richness ($\chi^2(1, N=51)=0.47$, $p=0.494$) [**Figure 4.3**]. No significant differences were observed for the rooms on the third floor of the Science and Arts buildings versus the first and second floor when observing fungal diversity, ($\chi^2(2, N=14)=0.88$, $p=0.648$) and ($\chi^2(2, N=14)=1.83$, $p=0.401$) respectively. Therefore, all of the rooms observed in these buildings were analyzed under the older building category. The older buildings had a higher fungal richness (3.1±1.3), when compared to the newer buildings (2.2±0.9) [**Figure 4.3A**]. The laboratories across the campus had a higher fungal diversity (3.2±0.9) when compared to offices (2.2±0.9) [**Figure 4.3B**]. No significant differences however were observed for the classrooms (2.9±1.4) compared to the laboratories and offices [**Figure 4.3B**]. Although the type of flooring did not have a significant effect on fungal diversity, carpeting (2.9±1.4) did show a favorable trend towards higher diversity compared to no carpeting cases (2.5±1.1) [**Figure 4.3C**].

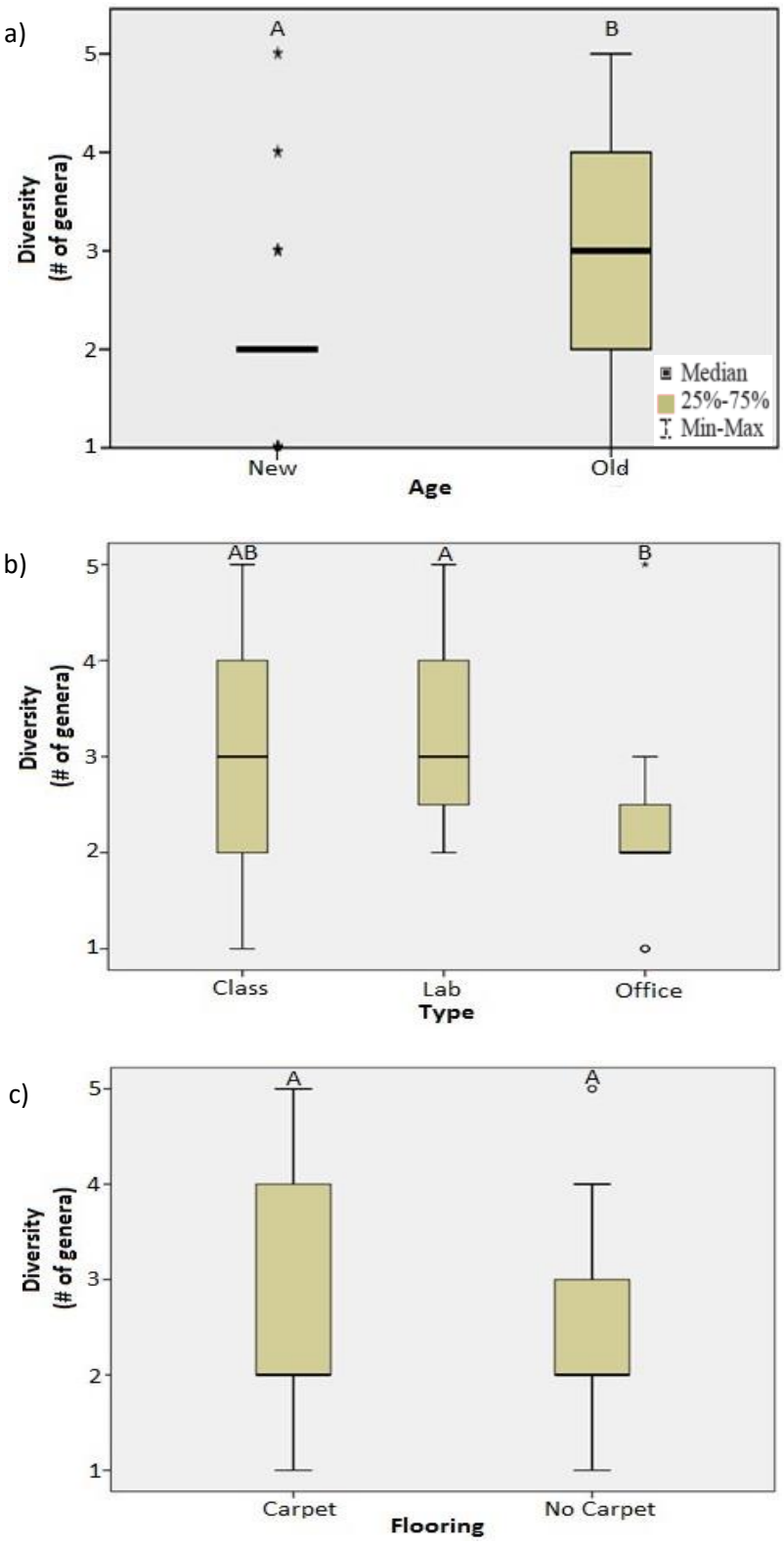


Figure 4.3 The relationship between indoor factors of (a) age, (b) type of room, and (c) type of flooring, influencing fungal diversity of drywall samples across 51 sampled rooms at UBCO. Different capital letters above boxplots indicate significant differences in diversity between the factors using Kruskal-Wallis and Dunn-Bonferroni post hoc tests ($p<0.05$).

4.3 Random environmental factors

The random effects from uncontrolled factors that varied from room to room, including temperature, humidity, occupancy level, presence of windows, and level of cleanliness were also analyzed [**Appendix F**]. A collective effect of these parameters was observed [**Appendix G**]. The average temperature of rooms was 22.4±0.2^{o}C, while the observed relative humidity was 38.5±1.6 % [**Table 4.2**]. Older buildings were found to have a slightly higher temperature and humidity (p=0.001, p=0.003) [**Table 4.2**; **Appendix A**]; and within buildings, although no significant differences in temperature and humidity were found between the rooms (p=0.626, p=0.068), the labs were found to have higher values [**Table 4.3**; **Appendix A**]. Despite these small differences, when tested separately, both of these random factors were correlated with the coverage of fungal growth and diversity of fungal communities on the drywall samples [**Table 4.4**]. Environments with higher temperature and humidity led to a higher coverage and diversity of fungi [**Table 4.4**]. However, it must be noted that these two random continuous variables were found to be highly confounded with some of the other random variables in the experimental space sampled (e.g., from **Appendix G** and **Table 4.4** notice the random confounding effects of both temperature and humidity with the dustiness factor). Hence, while collectively they clearly have a significant effect, statistically speaking it cannot be precisely identified which random factor has contributed to each response variable and to what extent. Similarly, occupancy ($\chi^2(2, N=51)=8.823$, $p=0.012$) and cleanliness ($\chi^2(2, N=51)=14.391$, $p=0.001$) level of rooms also affected the coverage [**Table 4.4**], and also show some level of confounding effect [**Appendix G**]. Fungal diversity response was also affected by occupancy $\chi^2(2, N=51)=9.073$, $p=0.011$ and cleanliness level $\chi^2(2, N=51)=13.445$, $p=0.001$ [**Table 4.4**], with higher dust presence leading to a higher diversity and also coverage. However, for occupancy, interestingly the maximum coverage and diversity were observed when occupancy level was at medium (labs) [**Appendix F**]. Comparing low and high levels of occupancy, it was observed that classrooms with a higher occupancy level and lower cleanliness levels led to a higher coverage and diversity compared to offices [**Appendix F**]. Presence of windows, however, was not a determining factor for coverage ($\chi^2(2, N=51)=0.303$, $p=0.582$) and diversity responses ($\chi^2(2, N=51)=0.070$, $p=0.792$) [**Table 4.4**].

Table 4.2 Average humidity and temperature measured for the four selected buildings at UBCO campus for the month of July 2014.

Building (age)	Temperature ^{o}C	Humidity %
ASC (new)	22.3 ± 0.2	38.3 ± 1.6
EME (new)	22.3 ± 0.2	37.3 ± 0.5
ART (old)	22.5 ± 0.2	39.2 ± 1.3
SC (old)	22.4 ± 0.2	38.9 ± 1.8
Average	22.4 ± 0.2	38.5 ± 1.6

Table 4.3 Summary of measurement of uncontrolled factors averaged for the three different types of rooms. Occupancy level was defined as low (<10), medium (10-20), and high (>20), while general dustiness level was defined as 1-very clean (low dustiness), 2-moderate, and 3-unclean (high dustiness).

Room type	Temperature (ºC)	Humidity (%)	Dustiness level (1-3)	Occupancy level	Window Presence
Class	22.36 ± 0.2	38.53 ± 1.0	1.95 ± 0.6	High=19 rooms	Yes=17, No=2
Lab	22.50 ± 0.2	39.64 ± 2.1	2.75 ± 0.5	Medium=12 rooms	Yes=12, No=0
Office	22.35 ± 0.2	37.9 ± 1.3	1.7 ± 0.8	Low=20 rooms	Yes=17, No=3

Table 4.4 The uncontrolled factors measured for all 51 rooms across the UBCO campus. The non-normal data for temperature and humidity (continuous variables) were tested for their correlation significance with fungal coverage and diversity of drywall samples using Spearman's rank correlation. The other non-normal factors (categorical) were analyzed for their significance using the Kruskal-Wallis test and Dunn-Bonferroni post hoc.

Factor	Coverage		Diversity	
	P-value	**Rho**	**P-value**	**Rho**
Temperature	P=0.000	0.745	P=0.000	0.529
Humidity	P=0.000	0.711	P=0.000	0.598
	P-value	χ^2	**P-value**	χ^2
Window presence	P=0.582	0.303	P=0.792	0.070
Dustiness level	P=0.001	14.391	P=0.001	13.445
Occupancy level	P=0.012	8.823	P=0.011	9.073

4.4 Weight loss testing

Analysis of the dry weights of drywall samples showed a significant weight loss over time, $F_{6,35}$=9810.2, p<0.05 [**Figure 4.4**]. The control samples that were only exposed to water with no fungal growth had an insignificant weight loss (12.7±0.10 %) compared to the control groups (12.4±0.2 %) [**Figure 4.4**]. The weight and physical property of drywall samples decreased as the % coverage of fungal growth on the drywall samples increased [**Figure 4.4**]. The highest coverage range of fungal growth on the drywall samples showed a 56.3±0.5 % decrease in weight loss [**Figure 4.4**].

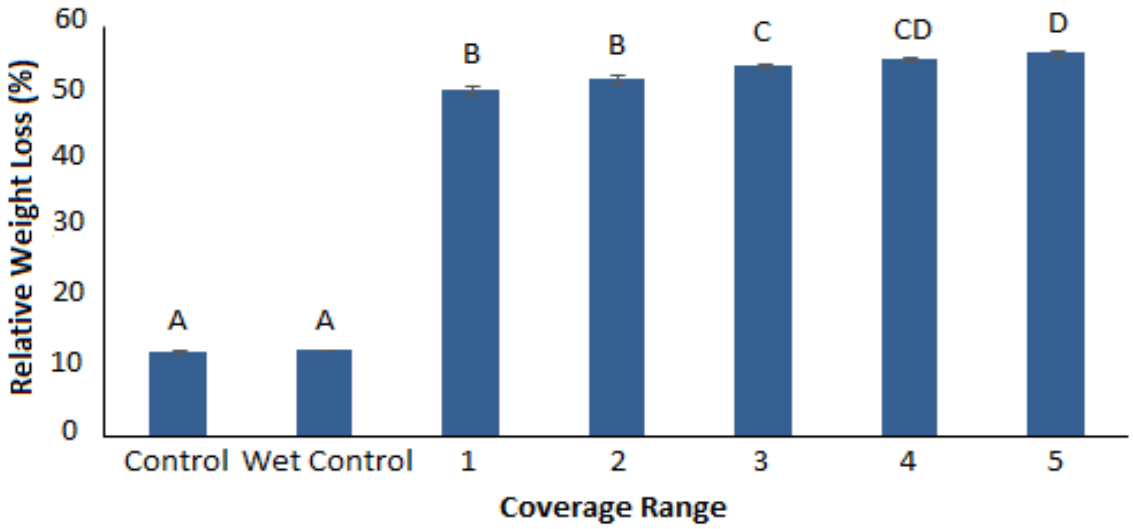


Figure 4.4 Weight loss of drywall samples with varying ranges (levels) of fungal growth coverage (1-5), over a one week time period (n=35). Control samples were not exposed to water or fungi, while wet control samples were only exposed to water. Error bars indicate standard deviation. Columns with different capital letters indicate significant differences in relative weight loss using ANOVA and Tamhane's post hoc tests ($F_{6,35}$=9810.2, p<0.05).

4.5 Mechanical testing

The physical and mechanical properties of the drywall samples were positively correlated (r= -.924, n=7, p<0.01) [**Appendix H**]. The growth of fungi on the drywall pieces had a significant effect on the mechanical properties of the drywall samples after tensile testing, $F_{6,31}$=396.3, p<0.05 [**Figure 4.5**]. The control samples that were only exposed to water with no fungal growth had a significant decrease in the maximum stress by 20% (8.2±0.4 MPa) when compared to the control groups (10.1±0.7 MPa) [**Figure 4.5**]. The tensile strength of the paper-backing of the drywall samples decreased as the % coverage of fungal growth on the drywall samples increased [**Figure 4.5**]. The highest % coverage range of fungal growth on the drywall samples showed a 86% decrease in the tensile strength property over time (1.4±0.2 MPa) compared to the control group [**Figure 4.5**].

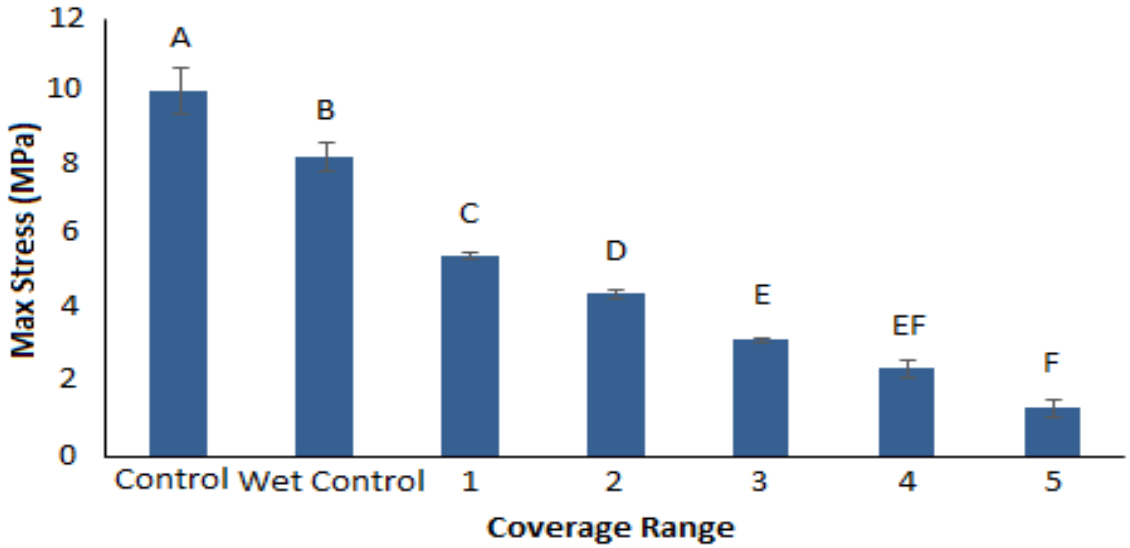


Figure 4.5 Tensile strength of drywall samples with varying ranges of fungal growth, post tensile testing (n=31). Control samples were not exposed to water or fungi, while wet control samples were only exposed to water. Error bars indicate standard deviation. Columns with different capital letters indicate significant differences in maximum stress using ANOVA and Tamhane's post hoc tests ($F_{6,31}$=396.3, $p<0.05$).

4.6 SEM imaging

The drywall samples exposed to high humidity and fungi led to the degradation of these building materials, which was evident by cracks in the paper-backing, presence of powdered gypsum in the glass jars, and the gypsum and paperback interface separating. **Figure 4.6 A-C** show SEM images of the control drywall samples, which are UV sterilized and contain no observed fungal growth on the surface of these materials. **Figure 4.6 D-F** show SEM images post tensile testing, with a clear visualization of spores and fungal hyphae. The fungi grew on the paper backing and gypsum under ambient conditions and utilized the carbon and nutrition sources. The damaged cracked fibers of cellulosic wood and fiber pull out due to tensile testing of these samples is also much greater compared to the control drywall samples [**Figure 4.6**].

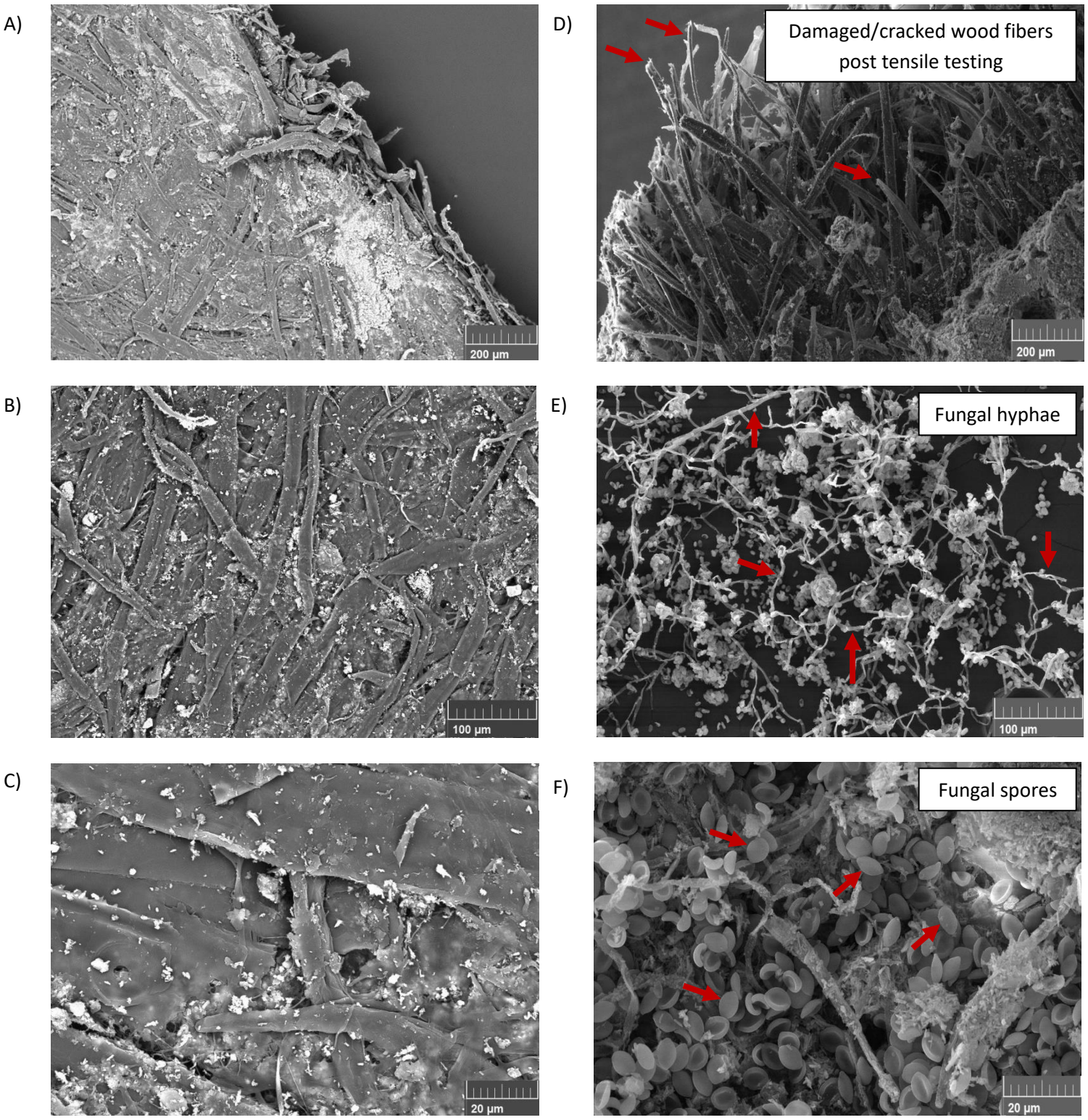


Figure 4.6 SEM micrographs of drywall samples showing (A-C) the control pieces which are UV sterilized and not exposed to dust and high humidity conditions, and (D-F) the drywall pieces post tensile testing and after 4 weeks of exposure to dust and high humidity conditions.

4.7 Generalized linear model results

Data analysis using the Generalized Linear Model (GzLM) produced very similar results to the non-parametric tests of Kruskal-Wallis presented earlier, when the main independent variables were employed separately in the regression model [**Table 4.5**]. However, when the independent variables of age, room type, and flooring were all entered together into one single GzLM multiple regression model, the p-values showed a significant effect of flooring on coverage, which is contrary to the previous result with Kruskal-Wallis [**Table 4.5**]. The flooring p-values in the multiple regression models, however, are still larger than the p-values of the age and type of room. When independent variables are combined together under a linear prediction model they are theoretically assumed to bring independent effects in an additive form (**Eqn. [2]**), which may explain why the results using the GzLM multiple regression model vary from the Kruskal-Wallis testing [McCullagh et al. 1989] for a few cases. Also it is important to note that the number of rooms with and without carpet has been most unbalanced in the current case study (with 20 carpet and 31 no-carpet rooms), when compared to other factors [**Appendix B**]. Another reason as to why the single GzLM would be more reliable in this case, is that there appears to be no collinearity between the controlled factors and thus there are no confounded effects between these variables [**Appendix G**].

Table 4.5 The p-values for three indoor factors of age, type of room, and flooring compared between the Kruskal-Wallis analysis with Dunn-Bonferroni post hoc tests, and the generalized linear model (GzLM).

Factors	**Coverage**			**Diversity**		
	GzLM-single factor	**GzLM-mutiple factor**	**K-W**	**GzLM-Single factor**	**GzLM-Multiple factor**	**K-W**
Age	0.000	0.000	0.000	0.008	0.007	0.004
Type	0.021	0.000	0.012	0.035	0.014	0.024
Flooring	0.545	0.025	0.601	0.918	0.101	0.494

Using the nested design model of the GzLM, age and type of room were again significant factors for the coverage and diversity of fungal growth on the drywall samples [**Table 4.6**]. Flooring was also found to be insignificant for coverage and diversity [**Table 4.6**]. Among different interaction terms, the type of room and flooring nested within the building age and was statistically significant for diversity, while no terms were found to be statistically significant for coverage responses [**Table 4.6**]. The former significance can also be observed via the interaction plots [**Appendix I**].

Table 4.6 Observed p-values for coverage and diversity using the nested GzLM model.

Factors	Coverage p-value	Diversity p-value
Age	.000	.000
Type	.000	.003
Flooring	.081	.100
Age*Type	.088	.007
Age*Flooring	.394	.003
Type*Flooring	.654	.706

4.8 Discussion

Building attributes and environmental conditions across the campus influenced the fungal growth and diversity observed on drywall. Dacarro et al. (2003) and Sivasubramini et al. (2004) suggested that age of buildings can have an important effect on fungal spore concentrations in the air. This was supported by the findings in this study, which showed that older buildings on campus had a higher concentration and diverse taxa of fungi in the air that led to a higher coverage on the drywall samples. Although the 18-19 year gap between the older buildings and newer buildings might not appear to be significant, the effects of building age are still evident and thus would strengthen if the gap were to be longer. It's also important to note that the later construction and renovation in the older buildings, which led to the addition of a third floor, did not lead to heterogeneity within the buildings. Therefore, although the first and second floors were 13 years older than the third floor in these two buildings, the rooms all showed a higher diversity of fungi compared to the rooms in the newer buildings and led to a higher fungal coverage on the drywall samples. The rooms in the aged buildings can also accumulate a higher dust level, e.g., due to older ventilation systems and less effective window insulations, which can cause more indoor fungal spores and lead to drywall biodegradation. Therefore, these buildings require diligent monitoring and prompt remediation in cases of water leakage and mould growth.

Older buildings also have antiquated construction materials and techniques. Hence, the observed high fungal coverage and diversity results may also be attributed to aged building materials, that can hold higher moisture levels [Sharpe et al. 2014], which is supported by the high humidity and temperature observed in the older buildings of this study. Some common building materials, such as solid lumber and bricks used in older buildings, however, can be advantageous compared to the gypsum boards used in newer homes. These building materials have a high saturation threshold for moisture, which can decrease condensation levels and make these materials less susceptible to fungal growth over time [Yan & Morrell 2015]. The newer buildings also have a different structural-frame compared to the older buildings. The concrete-frame of newer buildings have more CO2 and hydrocarbon emissions, while the steel-

frame of older buildings has more volatile organic compounds (VOCs) and heavy metal emissions [Guggemos & Harvath 2005]. Older steel-framed buildings also have higher thermal conductivity and heat transfer, which can create higher temperatures in these buildings as supported by this study, and thus have a higher energy consumption and environmental emissions of air conditioning [Xing et al. 2008]. A related point to consider is that the older buildings on this campus have atria for studying and relaxation purposes, with one enclosed environment and one exposed to the outdoor and weather conditions. Plants in this atria can also increase humidity and affect the fungal diversity seen in these older buildings [Sharpe et al. 2014]. Although all buildings on this campus are tested regularly and fully inspected, it is important to note that indoor mould growth can be hidden in pipes, wall cavities, above ceilings, inside air ventilation systems, behind wallpapers, under carpets, and many more environments.

When observing the different types of rooms and their influence on fungal growth and diversity on drywall, classrooms with the highest occupancy level, showed a higher coverage and diversity compared to offices with low occupancy levels. People in high occupancy environments can create higher dust levels that enable microorganisms to grow [Brown et al. 1996], as supported by our results, transport more bioaersols from outdoor environments [Muilenberg 1995], and also increase humidity and temperature in indoor spaces [Sharpe et al. 2014]. The increased air velocity current by the movement of occupants can also release more spores [Cabral 2010; Frankel et al. 2014]. Although labs had a medium occupancy level, they also had a higher fungal coverage and diversity response compared to the offices. The increased activity level of the labs' occupants from conducted experiments, and usage of materials and equipment can create high dust levels. Sources of fungi in labs vary from field samples, animal testing and by-products, purchased materials that have accumulated fungal spores over time, to the experimenters themselves. In addition to these effects, it is also important to note that labs and classrooms have heating ventilation and air conditioning (HVAC) systems that have a higher air change rate. This greater airflow can lead to an increased concentration of spores in these environments and as a result increase fungal growth and diversity [Rogers 2003; Dannemiller et al. 2016]. Lab rooms also had windows that remained closed the majority of the time, which can lead to poor air circulation and increase humidity and temperature in these types of rooms [Jamriska et al. 2000]. Overall the presence of windows was not a significant factor in affecting fungal growth and diversity on drywall; however, it is important to note that there were only 5 rooms out of the 51 sampled that had no windows, so a balanced comparison should be made and evaluated in future studies.

The factor of flooring had no significant effect on the fungal growth and diversity observed on the drywall samples compared to the two other controlled variables. However, carpeting did show a favourable trend towards slightly higher percent coverage and diversity of fungi; which

is in support of the findings by Sharpe et al. (2014) and Wani et al. (2014). Carpets can provide a habitat to support fungal growth even without moisture damage and increase indoor populations of fungi [Kemp et al. 2002]. In contrast, Chew et al. (2003) also found that although carpets contained higher dust-borne fungal concentration than non-carpet rooms, it didn't lead to higher levels of airborne fungi in those environments. A higher number of classrooms sampled in the newly designed buildings on campus are non-carpeted rooms compared to the older buildings. This trend of moving towards non-carpet flooring, however, is not observed for the offices in these environments, which have mostly carpet flooring in older and newer buildings. Carpets are also sources of VOCs in indoor air and have absorptive capacities that allow them to harbor allergens, dust, and microorganisms and re-emission over prolonged periods of time [Won et al. 2000; Zock etl al. 2002]. Rooms with carpeting can thus affect indoor air quality and occupants' health. Preventative measures such as using disinfectants and vacuuming [Vojta et al. 2001], and moving towards the removal of carpeting in rooms can decrease allergens in indoor environments.

After evaluating factors that affect fungal diversity on drywall, determining what is in fact growing on these drywall pieces is essential. The most commonly occurring species found in the present study are consistent with reports on the species composition of dust [Bronswijk 1981; Cabral 2010]. Namely, the results of this study displayed an abundant presence *of Aspergillus*, *Penicillium,* and *Cladiosporium* spp., and a lower abundance of *Alternaria, Stachybotrys, and Fusarium* spp. found in the atmosphere. This observation is consistent with those of past published research [Cabral 2010; Adan & Samson 2011], which suggested that the most abundant species found produce small, light spores in comparison to those found less abundantly which produce fewer, bigger, and heavier spores that aren't easily airborne. The abundance of the smaller spores can also be more problematic and cause allergenic responses, since they can penetrate into the alveolar region of the lungs when inhaled [Cabral 2010]. Our results suggest that environmental conditions and characteristics unique to each type of room within buildings, can exert influence on microbial communities available in that environment. From all of the observed fungal taxa across the campus, *Alternaria alternata*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus versicolor*, and *Stachybotrys chartarum* were found in rooms of the older buildings. *Stachybotrys* spp. are most often associated with moist building conditions [Grant et al. 1989; Andersen et al. 2011], which explains why their spores were most often found in older buildings that had higher humidity conditions. The presence of *Alternaria* spp. in older buildings is also higher, since they have higher humidity conditions and more rooms sampled with carpeted flooring, which can increase this type of fungi [Wani et al. 2014]. It is also important to note that *Stachybotrys* spp. produce spores in wet slimy heads and are not readily airborne [Andersen et al. 2002; Adan & Samson 2011], and thus their presence might be just as prevalent in newer buildings and not detected due to dust collection methods

applied in this study. No significant differences in fungal composition were observed between the type of rooms and flooring, which shows that these fungal spores are ubiquitous.

When observing the mould on the drywall samples for fungi hazardous to human health, five fungal species (*Aspergillus niger*, *A. fumigatus*, *A. versicolor*, *Alternaria alternata*, and *Stachybotrys chartarum*) that are known to be human pathogens were also detected and present only in older buildings. Most fungal species present in indoor environments come from outdoor sources [Cabral 2010; Rogers 2003]. Although only a fraction of fungal species present in outdoor environments have been detected in indoor environments [Adan & Samson 2011], numerous health effects have been attributed to these indoor fungi [Cabral 2010; Douwes et al. 2003; Mihinova & Pieckova 2012; Andersen et al. 2002]. The presence of these pathogenic fungal species can lead to mycotoxins and VOCs, which can increase the prevalence of diseases [Wani et al. 2014; Nielsen 2003]. Exposure to the allergenic fungi *Alternaria alternatata* can induce skin and pulmonary infections [Grant et al. 1989; Sharpe et al. 2014]. The mycotoxins produced by these fungal species such as ochratoxin, gliotoxin, sterigmatocystin, and trichothecenes can have health effects such as Aspergiollis, decreasing immune function, affecting the liver and kidneys, affect CNS functioning, and can even be carcinogenic [Ababutain 2013; Andersen et al. 2002; Brown et al. 1996; Nielsen 2003]. *Stachybotrys chartarum*, commonly known as black mould, can lead to sick building syndrome (SBS), which can lead to unpleasant odors in indoor environments and symptoms such as headaches, dizziness, fatigue, and difficulty concentrating [Betancourt et al. 2013; Karunasena et al. 2000]. Indoor mould growth can affect the health of occupants living in houses, studying in school and institutions, and working environments. This can have a major economic impact and increase healthcare costs, building repair costs, and business costs [Fisk et al. 2007; Mudarri 2016]. An important factor to state is that this study was an extreme case of high humidity conditions and although these fungal species were found to be present in indoor environments such as the campus buildings, exposure levels of the airborne microorganisms were not measured.

It was hypothesized that degradation by fungal growth can change the structure of the paper backing of drywall with a negative impact on their physical and mechanical properties. The results of the study clearly indicated that degradation by microorganisms can decrease both physical and mechanical properties of these materials, as shown by the increased weight loss and decreased tensile strength of test samples. The increased moisture exposure and fungi can create voids between the bondage and attachment of gypsum and paper, and as a consequence lead to extensive de-bonding and reduction of the material load carrying capacity. This is supported by a study from Canada Housing and Mortgage Corporation that found a 0-2% decrease in the flexural strength and an increase in moisture content by 5% caused gypsum panels to crumble [CMHC 2007]. Porosity of the gypsum can also decrease mechanical properties of drywall materials [Chen et al. 2010], since they have high water holding

capabilities which can lead to fungal growth [Dedesko & Siegel 2015]. This high moisture retention can additionally lead to the aggregation of gypsum crystals which reduces contact with neighbouring crystals and lowers the structural efficiency of the material [Chen et al. 2010]. Mechanical properties of drywall materials are important for building designers, as they are frequently used in construction, and thus evaluating how microorganisms around us affect these indoor materials is crucial.

With increasing awareness and monitoring of indoor fungal growth, new preventative measures and physical removal of these microorganisms is becoming more and more important. Choosing less susceptible building materials (e.g., glass fiber), and manufacturing materials that are non-toxic and water resistant can prevent microbial growth in indoor environments. Antimicrobial paints and different coatings may also be used to prevent microbial growth. A study by Krause et al. (2006) found that Kilz Premium sealer and Foster 40-20 coating can be used to sanitize and clean mould growth on drywall. Physical disinfection techniques, such as UV exposure, and the application and usage of general biocides and antimicrobial agents, which suppress microbial growth, can also be recommended [Cole & Foarde 1999]. It has been observed, however, that biocide treatments can only offer partial resistance to fungal growth on building materials [Vacher et al. 2010], with the killing of spores but the remains of allergenic particles [Krause et al. 2006]. Therefore, further research is required to understand indoor environments in order to prevent mould growth and to find new methods of mould removal on indoor material.

Chapter 5. Conclusions and Future Work

5.1 Conclusions

Although there have been a substantial number of studies on indoor factors affecting fungal growth on building materials, the underlying mechanism of such associations are not well understood and still need further experimental confirmation. The results of this case study suggested that older buildings can support a higher diversity of fungi and lead to more fungal growth on drywall material. Therefore, a more diligent monitoring of older buildings may be critical (e.g., as a proactive measure to potential flooding, etc.), since fungal species hazardous to health were also discovered in some old buildings. Different types of rooms can support the growth of a variety of different fungal species, with laboratories, classrooms, and offices in order having more fungal growth and diversity of species observed on drywall. Proper ventilation in these rooms (especially labs) and frequent cleaning strategies to decrease dust can help with prevention of fungal growth. The type of flooring (presence of carpet) was not a significant indoor factor influencing the amount of fungal growth and diversity, when compared to the age and room type. The role of fungi in drywall degradation was strongly supported by the material tests in the study, which showed that these microorganisms can affect the mechanical constants and structural integrity of the drywall material over time. Increasingly, we are gaining understanding of the complexity of fungi in indoor environments and their effects on human health and building material integrity. This increased insight can eventually enable optimization and usage of new building materials, help with design and functionality of new buildings, and decrease health concerns.

5.2 Future work recommendation

Gypsum boards are a major component of houses. Microbial growth, such as fungi, on these materials can be a devastating and problematic issue. Mould growth can lead to costly remediation and cleaning products, and even the removal of entire drywall. Therefore, understanding factors influencing their growth is critical for drywall producers, construction workers, building designers, and home owners.

The present research results assisted in enhancing our understanding of some factors influencing fungal growth on these building materials. The significant identified factors in this study, however, were only a fraction of the available possibilities and indoor variations that can affect growth and diversity of indoor biota. Therefore, further research is needed to understand associations/interactions of built environment biota with different engineered materials in a broader sense. In particular, further research is needed for a) manufacturing new materials that are less susceptible to fungal growth, b) fast characterization and detection methods specifically for fungal species that are problematic for human health, c) finding non-toxic and

cost effective cleaning products to remove fungi on building materials (i.e., for maintenance purposes), and d) investigating associations between these organisms as well as volatile organic compound emissions from indoor building materials (i.e. due to their biodegradation process), and health of occupants.

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Appendices

Appendix A- Supplementary figures of the correlation between uncontrolled factors and the controlled factors of age, type, and flooring

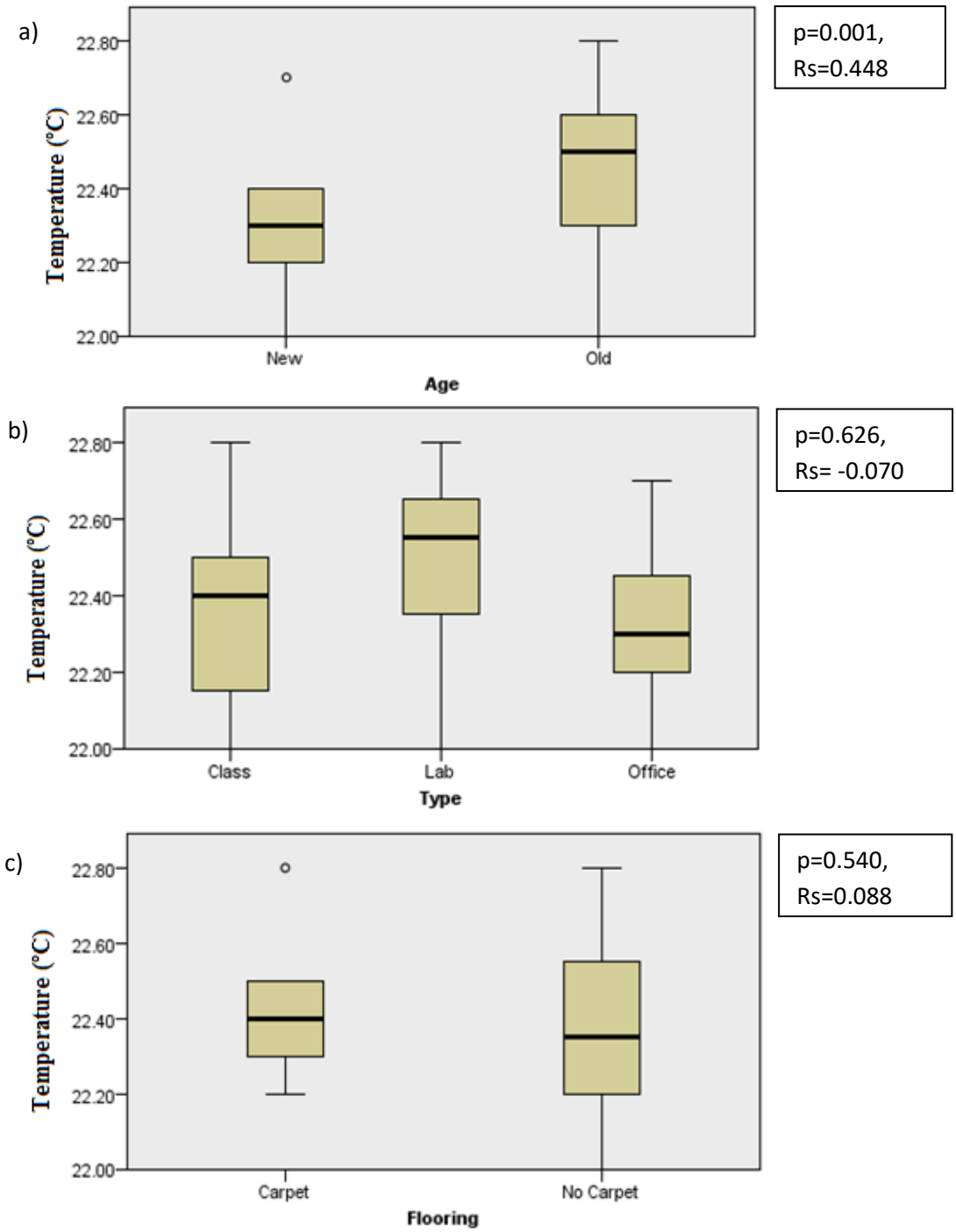


Figure A.1 The uncontrolled factor of temperature measured for the controlled factors of age, type, and flooring.

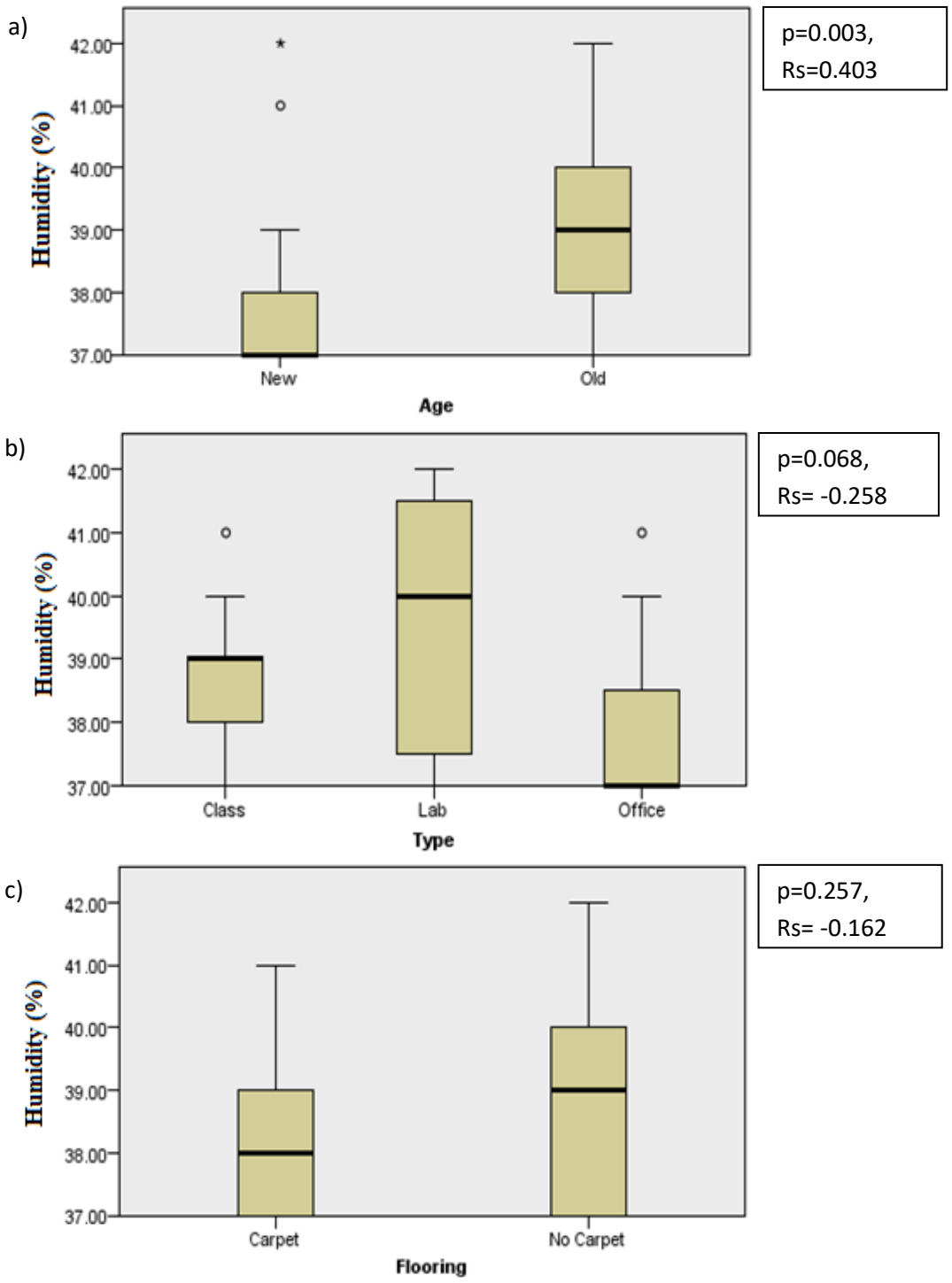


Figure A.2 The uncontrolled factor of humidity measured for the controlled factors of age, type, and flooring.

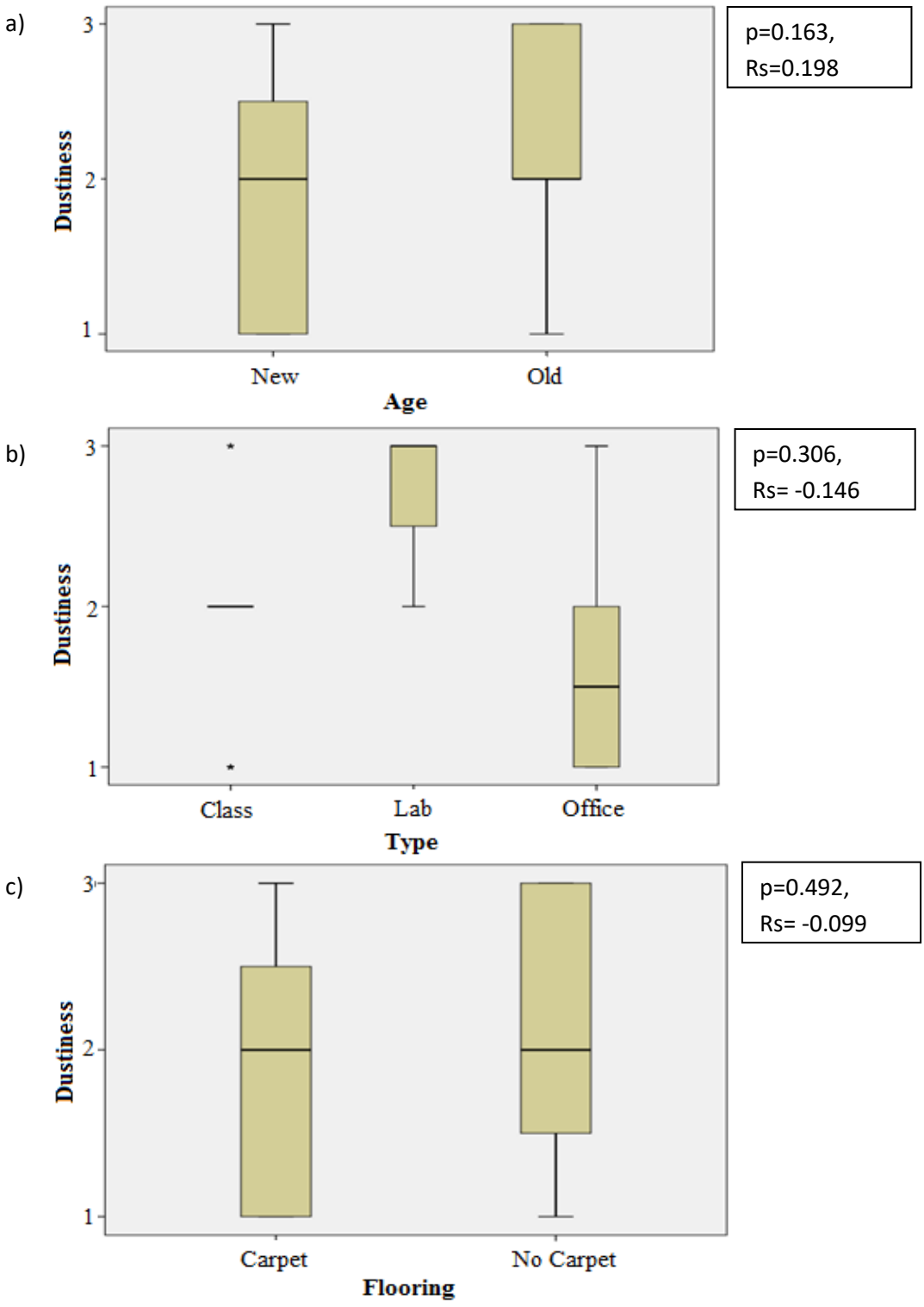


Figure A.3 The uncontrolled factor of dustiness measured for the controlled factors of age, type, and flooring.

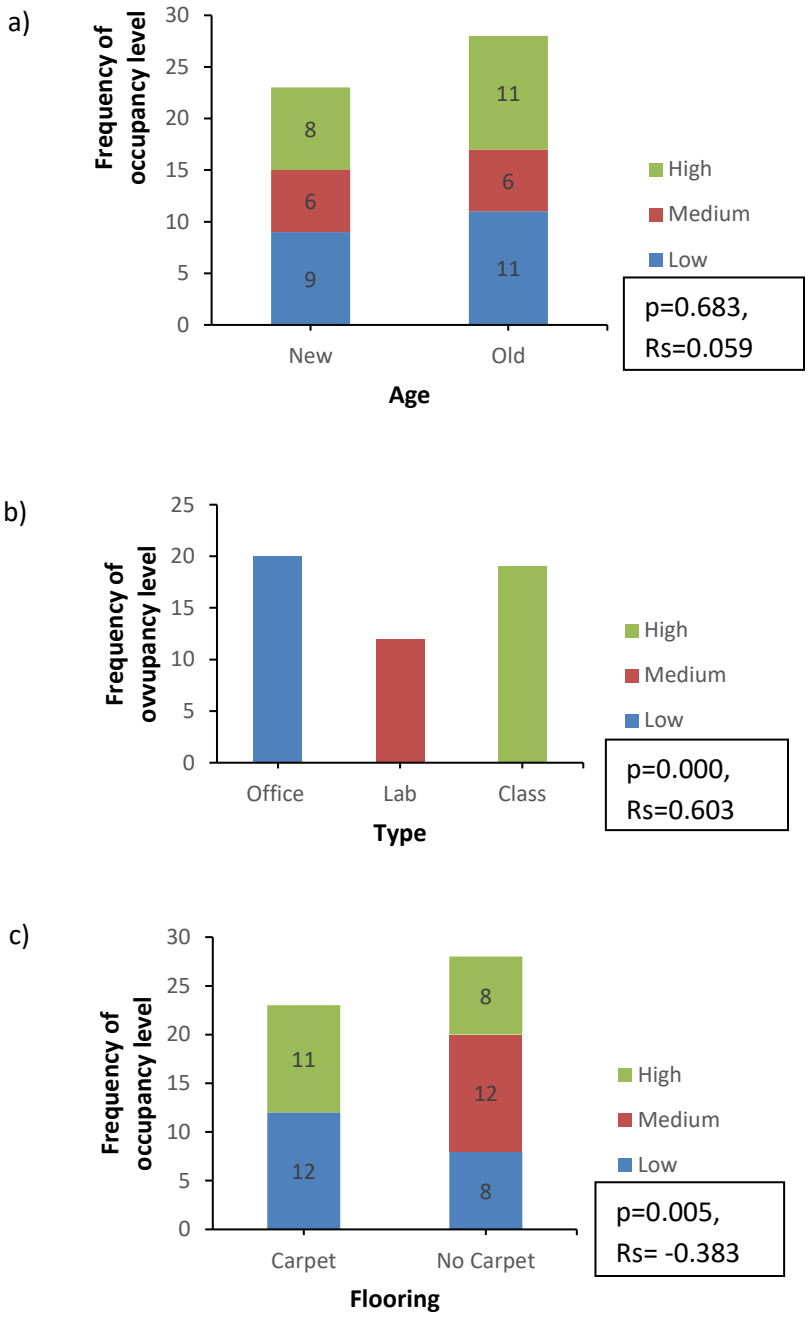


Figure A.4 The uncontrolled factor of occupancy level measured for the controlled factors of age, type, and flooring.

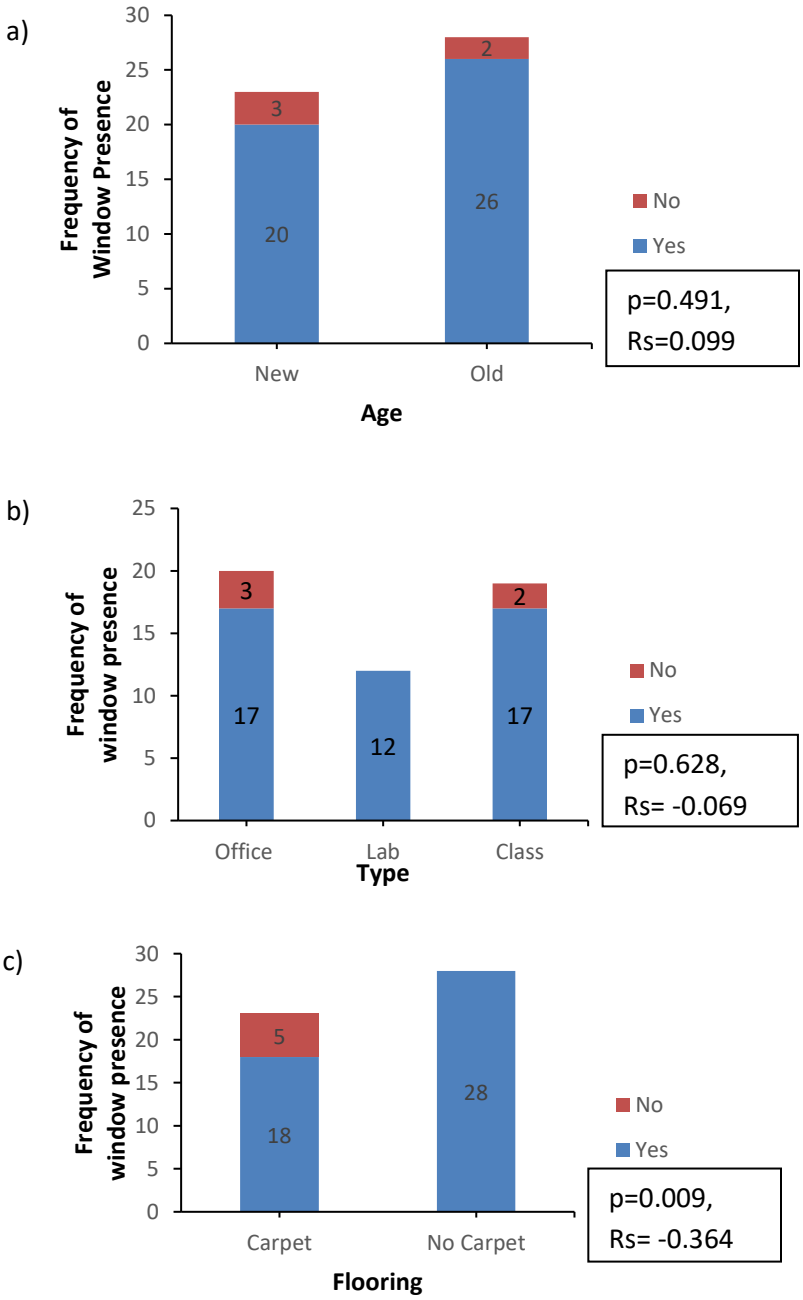


Figure A.5 The uncontrolled factor of window presence measured for the controlled factors of age, type, and flooring.

Appendix B- Supplementary table for number and specification of rooms sampled

Table B.1 Summary of number of rooms and their location as sampled.

Building (age)	Office		Laboratory		Classroom	
	Carpet	No Carpet	Carpet	No Carpet	Carpet	No Carpet
EME (new)	3	0	0	3	0	3
ASC (new)	3	3	0	3	2	3
ART (old)	3	3	0	3	3	2
SCI (old)	3	2	0	3	3	3
Sum	12	8	0	12	8	11

(a) **(b)**

Figure B.1 Photographs of the atria in (a) Arts and (b) Science building at UBCO.

Appendix C- Supplementary table and figure for sample normality assumption test

Table C.1 Example of tested normality assumption for the flooring factor when observing fungal diversity.

Response	Shapiro-Wilk Test		
	Statistic	**Df**	**Sig.**
Diversity	.881	51	.000

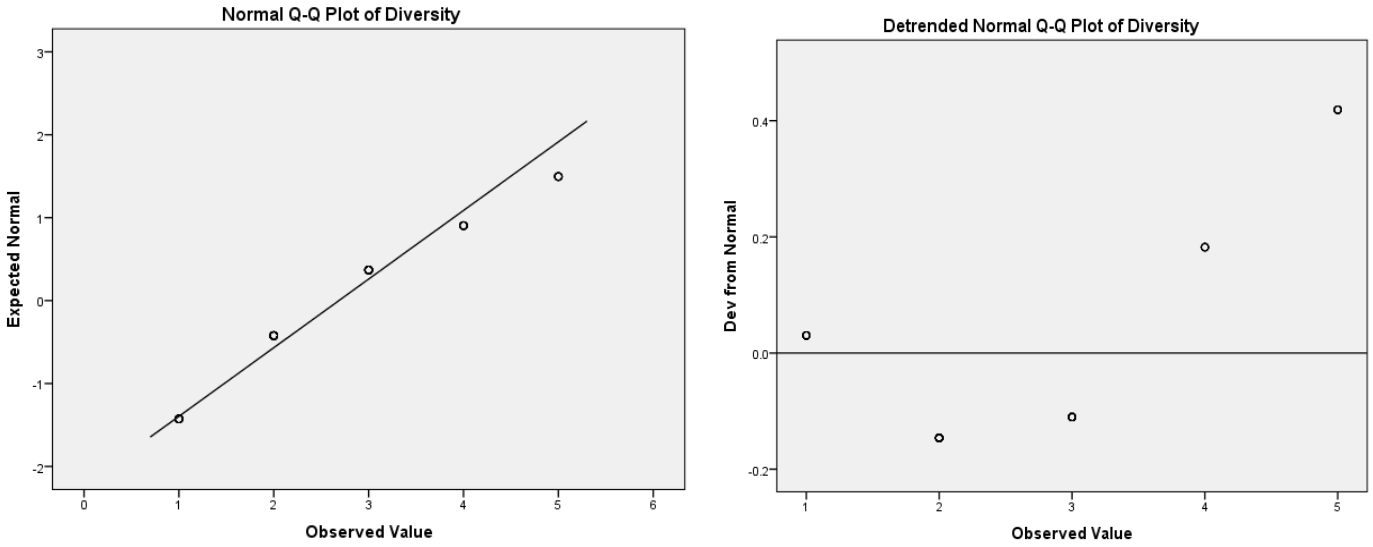
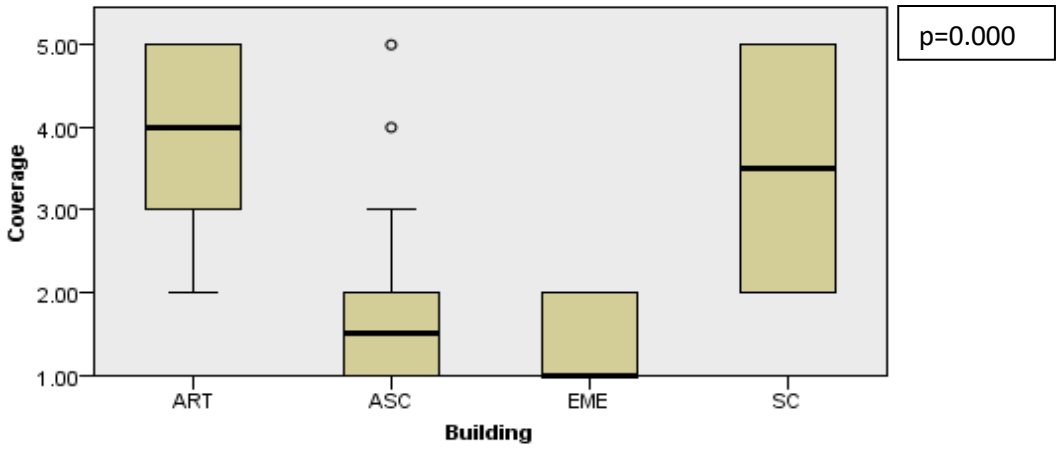


Figure C.1 (A) The normality Q-Q plots and (B) detrended normal Q-Q plots for diversity. A value less than 0.05 in the Shapiro-Wilk test shows the non-normality. Also note that each point represents multiple rooms (given ordinal response variable).

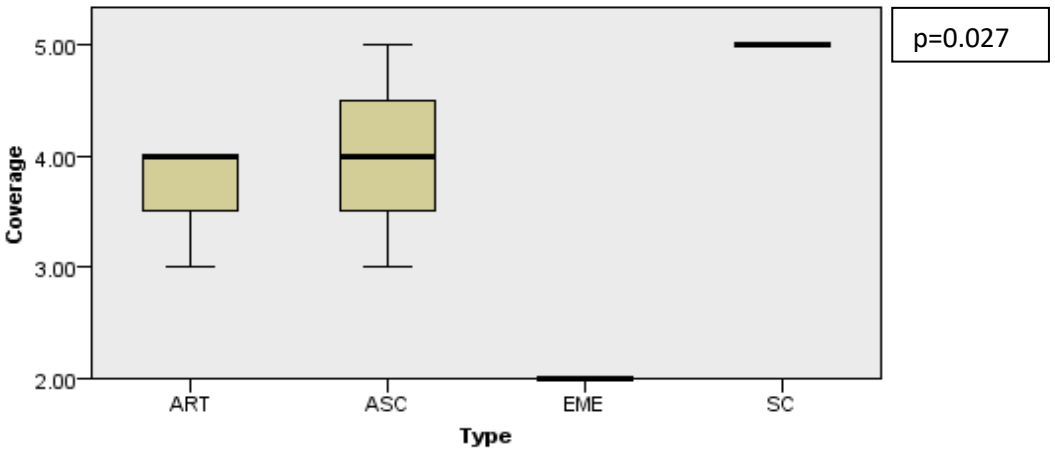
Appendix D- Supplementary table and figures for the nested design analysis

Table D.1 Example of the generalized linear model output using the nested design for fungal coverage on the drywall samples and the three indoor factors of building age, floor type, and flooring type.

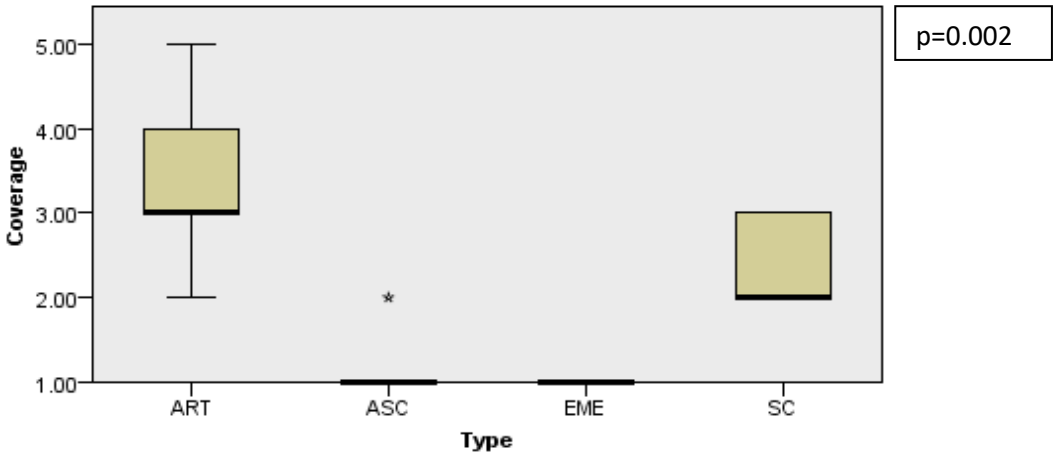
				Hypothesis Test		
Parameter		**B**	**Std. Error**	**Wald Chi-Square**	**Df**	**Sig.**
Threshold	[Coverage=5.00]	-3.707	1.0842	11.690	1	.001
	[Coverage=4.00]	-2.189	.9780	5.011	1	.025
	[Coverage=3.00]	-.577	.8692	.440	1	.507
	[Coverage=2.00]	33.886	5408.8192	.000	1	.995
[Age=New]		54.033	8512.1940	.000	1	.995
[Age=Old]		0[b]	.	.	.	.
[Flooring=Carpet]		-1.819	1.1613	2.452	1	.117
[Flooring=No carpet]		0[b]	.	.	.	.
[Type=Class]		-2.388	1.2569	3.608	1	.057
[Type=Lab]		-3.754	1.2800	8.601	1	.003
[Type=Office]		0[b]	.	.	.	.
[Age=New] * [Type=Class]		-16.150	3794.8270	.000	1	.997
[Age=New] * [Type=Lab]		-51.273	8512.1941	.000	1	.995
[Age=New] * [Type=Office]		0[b]	.	.	.	.
[Age=Old] * [Type=Class]		0[b]	.	.	.	.
[Age=Old] * [Type=Lab]		0[b]	.	.	.	.
[Age=Old] * [Type=Office]		0[b]	.	.	.	.
[Flooring=Carpet] * [Type=Class]		-.328	1.6540	.039	1	.843
[Flooring=Carpet] * [Type=Office]		0[b]	.	.	.	.
[Flooring=No carpet] * [Type=Class]		0[b]	.	.	.	.
[Flooring=No carpet] * [Type=Lab]		0[b]	.	.	.	.
[Flooring=No carpet] * [Type=Office]		0[b]	.	.	.	.
[Flooring=Carpet] * [Age=New]		-16.719	3794.8270	.000	1	.996
[Flooring=Carpet] * [Age=Old]		0[b]	.	.	.	.
[Flooring=No carpet] * [Age=New]		0[b]	.	.	.	.
[Flooring=No carpet] * [Age=Old]		0[b]	.	.	.	.
(Scale)		1[c]				



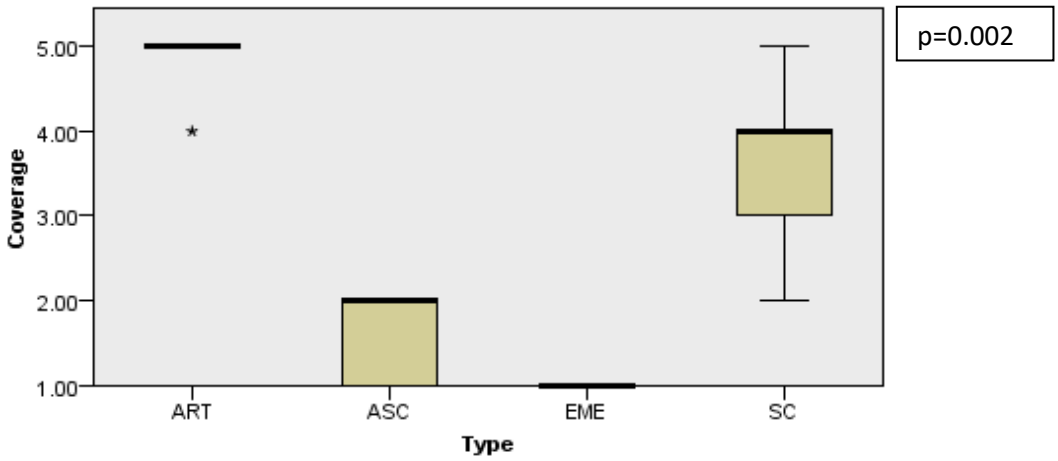
(a) Fungal coverage compared between all four buildings tested



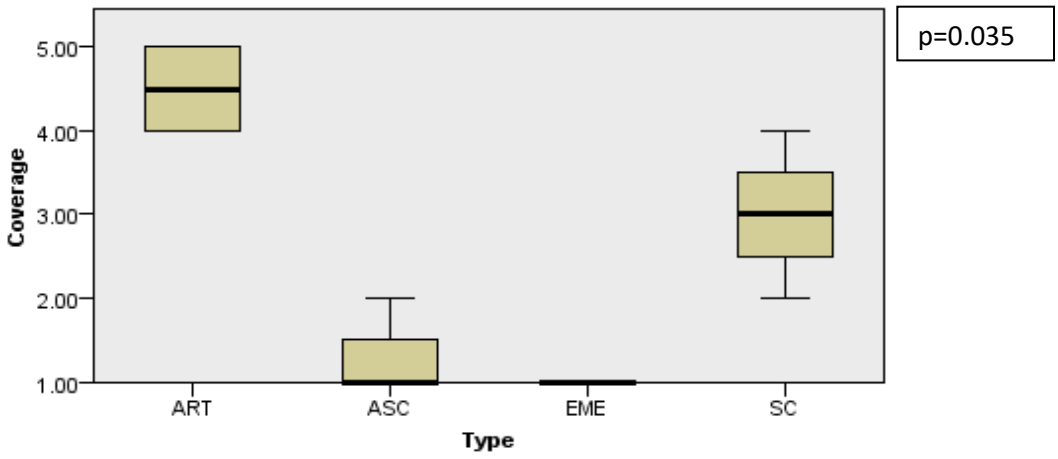
(b) Fungal coverage compared for laboratories between all four buildings tested



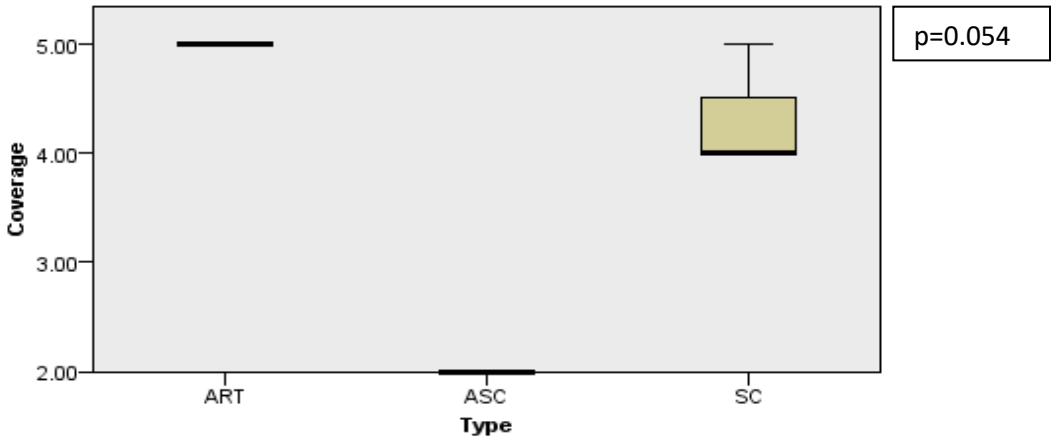
(c) Fungal coverage compared for offices between all four buildings tested



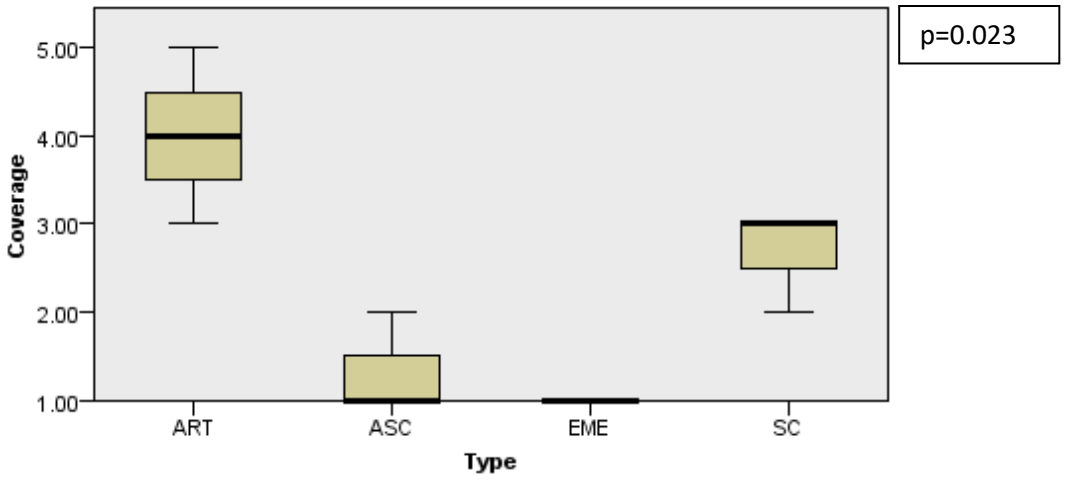
(d) Fungal coverage compared for <u>classes</u> between all four buildings tested



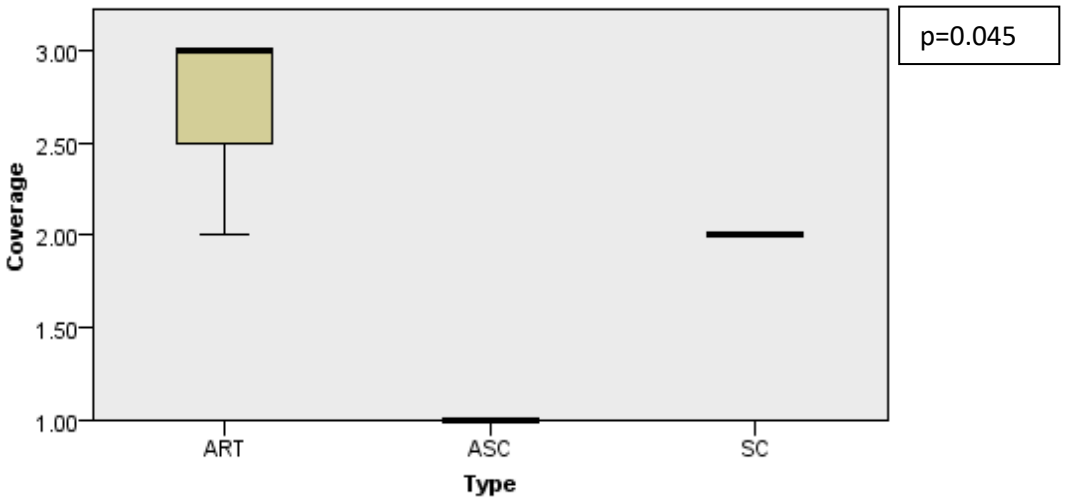
(e) Fungal coverage compared for <u>classes with no carpets</u> between all four buildings tested



(f) Fungal coverage compared for <u>classes with carpets</u> between all four buildings tested



(g) Fungal coverage compared for <u>offices with carpets</u> between all four buildings tested



(h) Fungal coverage compared for <u>offices with no carpets</u> between all four buildings tested

Figure D.1 Comparing details of all the four buildings on campus with different types of rooms and flooring when testing for fungal coverage on drywall samples.

Appendix E- Supplementary table and figure for the rank correlation between coverage and diversity

Table E.1 The correlation table between fungal diversity and fungal coverage on the drywall samples (N=51).

		Coverage	Diversity
Coverage	**Correlation Coefficient**	1.000	.737**
	Sig. (2-tailed)		.000
Diversity	**Correlation Coefficient**	.737**	1.000
	Sig. (2-tailed)	.000	.

** Correlation significant at the e 0.01 level

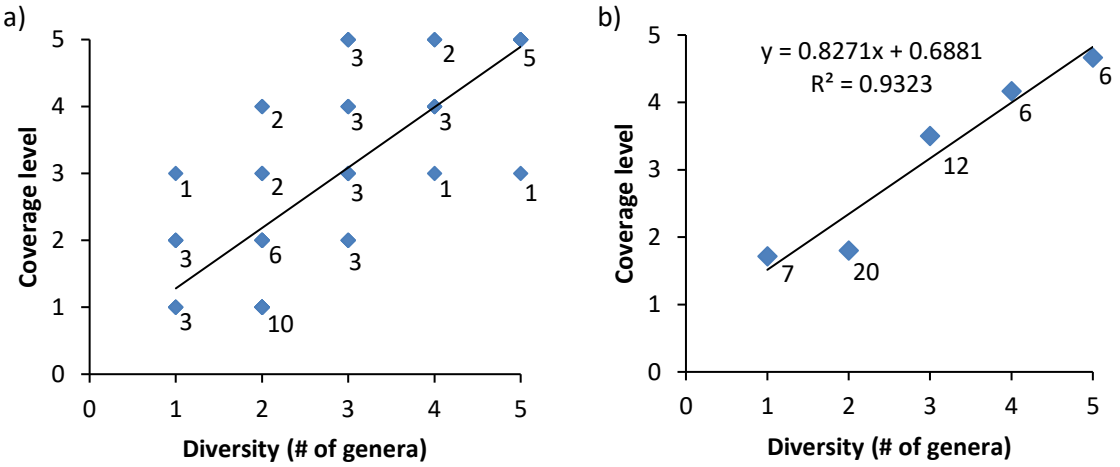
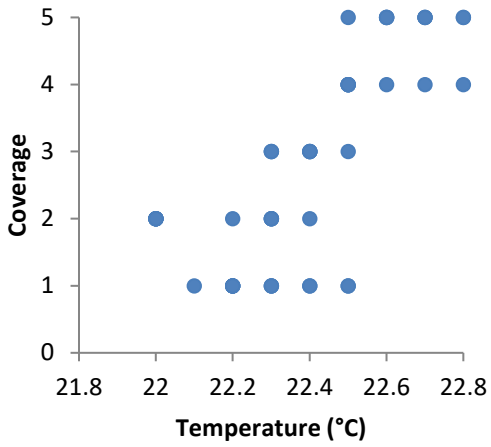
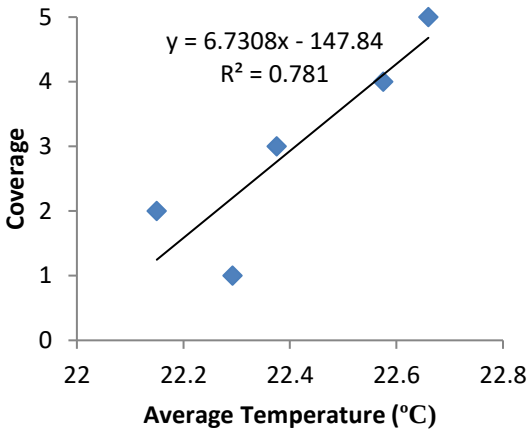


Figure E.1 Visualization of high correlation between fungal coverage and fungal diversity a) for all 51 rooms, and b) the average of all 51 rooms. Each data point represents multiple rooms as displayed with numbers by each point.

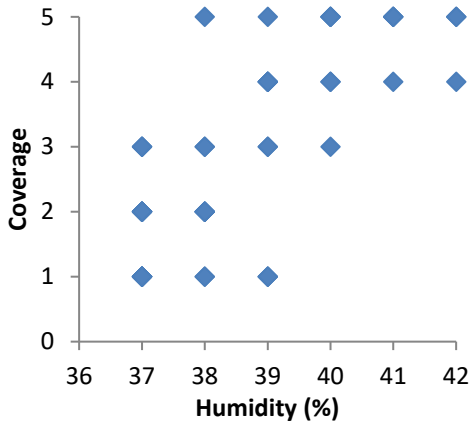
Appendix F- Supplementary figures for the uncontrolled factors affecting coverage and diversity

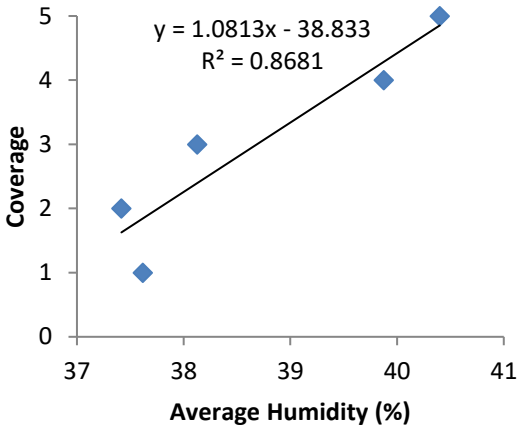
a)



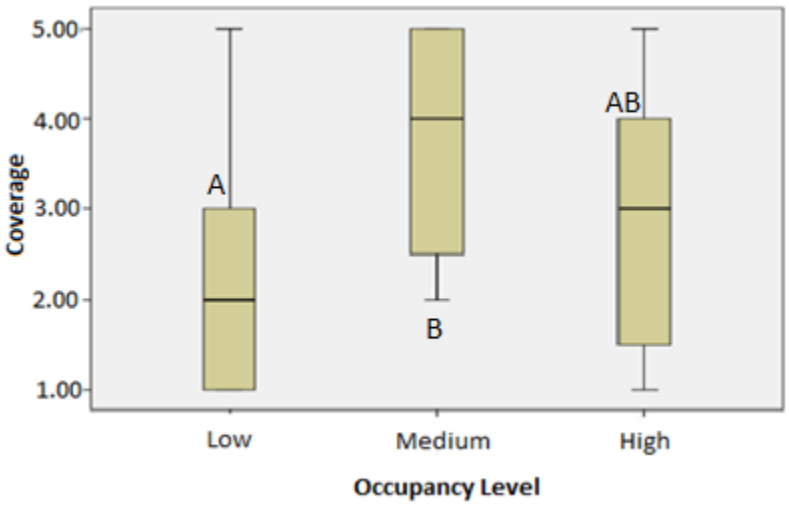


b)





c)



p=0.012

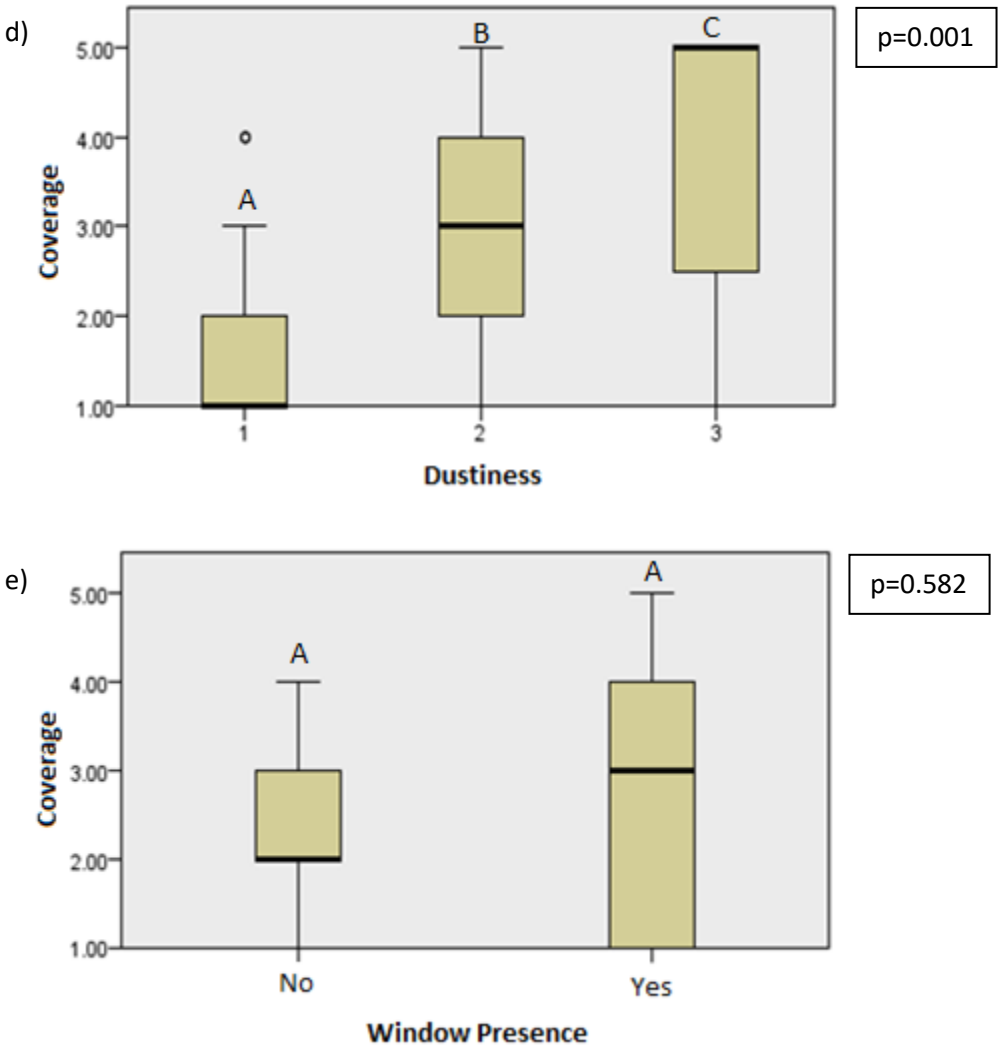
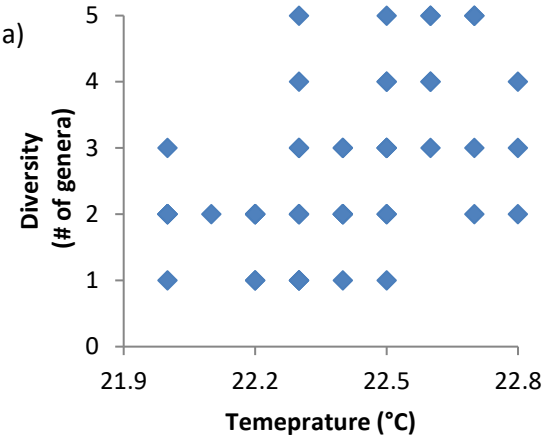
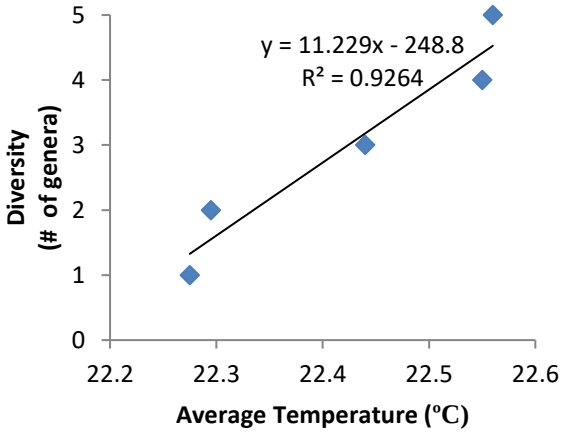


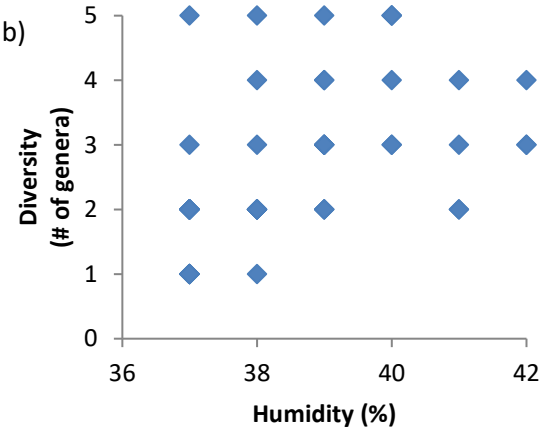
Figure F.1 The uncontrolled factors of temperature, humidity, occupancy level, dustiness, and window presence affecting fungal coverage on drywall. Different capital letters above boxplots indicate significant differences in fungal coverage between the factors using Kruskal-Wallis and Dunn-Bonferroni post hoc tests ($p<0.05$).

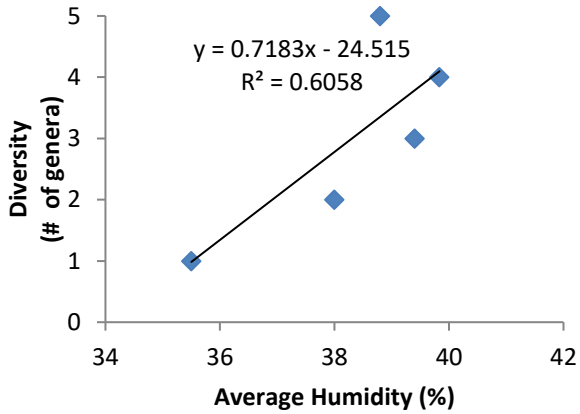
a)


Diversity (# of genera)
0
1
2
3
4
5
21.9
22.2
22.5
22.8
Temeprature (°C)

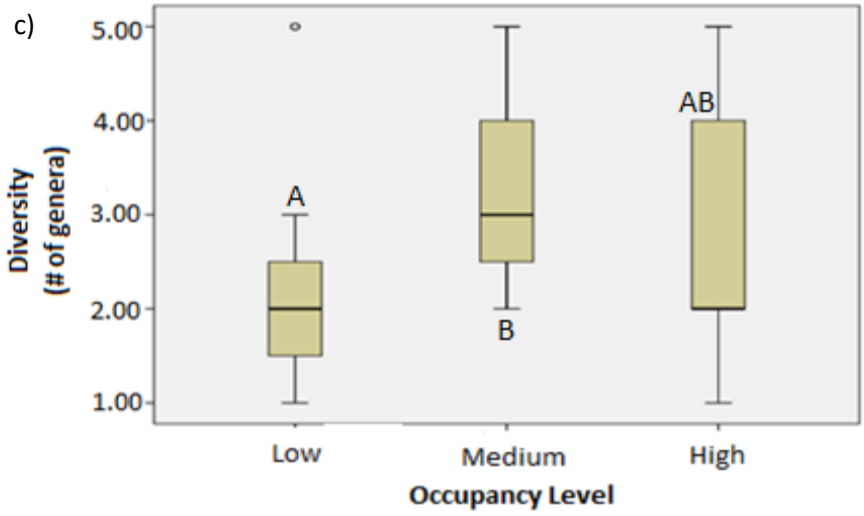

y = 11.229x - 248.8
R² = 0.9264
Diversity (# of genera)
0
1
2
3
4
5
22.2
22.3
22.4
22.5
22.6
Average Temperature (°C)

b)


Diversity (# of genera)
0
1
2
3
4
5
36
38
40
42
Humidity (%)


y = 0.7183x - 24.515
R² = 0.6058
Diversity (# of genera)
0
1
2
3
4
5
34
36
38
40
42
Average Humidity (%)

c)


Diversity (# of genera)
1.00
2.00
3.00
4.00
5.00
A
B
AB
Low
Medium
High
Occupancy Level

p=0.011

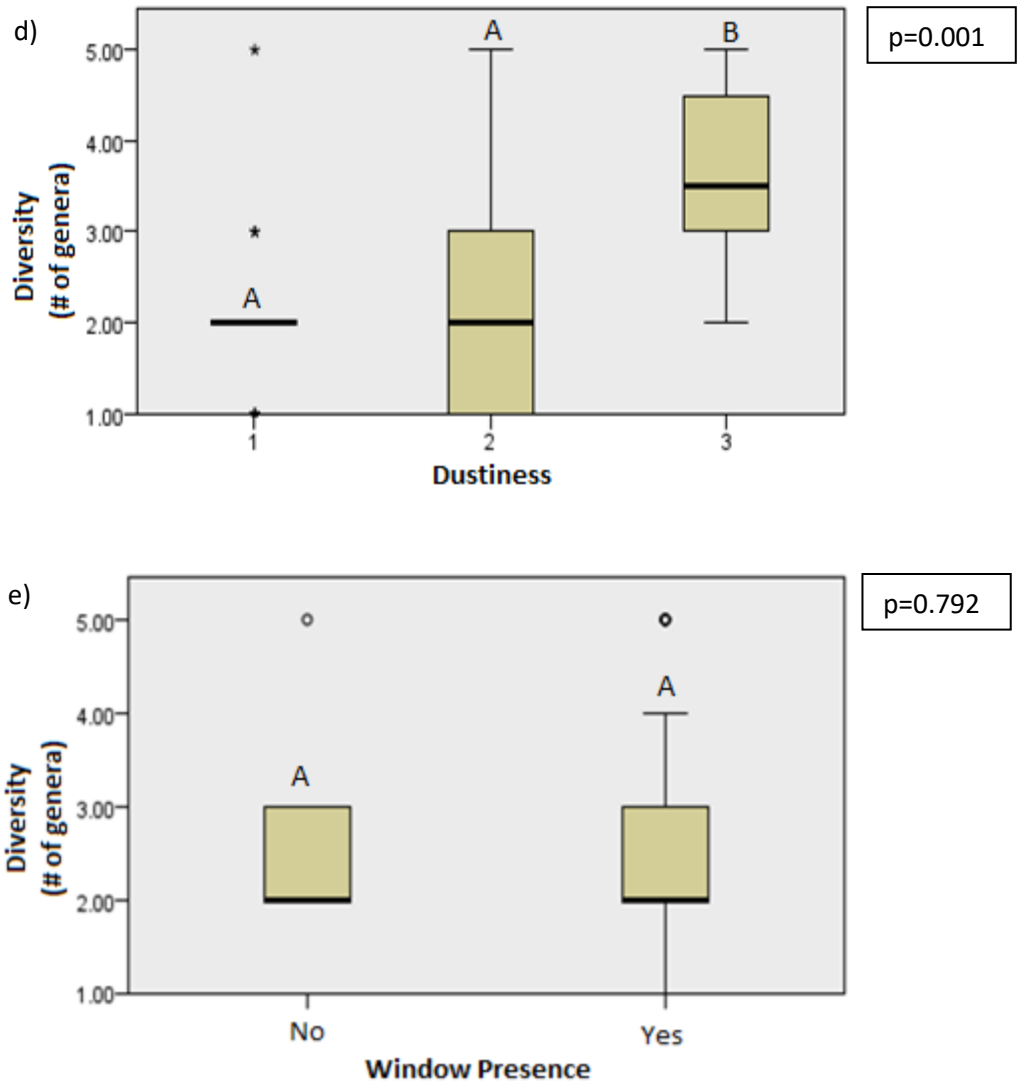


Figure F.2 The uncontrolled factors of temperature, humidity, occupancy level, dustiness, and window presence affecting fungal diversity on drywall. Different capital letters above boxplots indicate significant differences in fungal diversity between the factors using Kruskal-Wallis and Dunn-Bonferroni post hoc tests ($p<0.05$).

Appendix G- Supplementary table and figures assessing confounding effects among controlled factors and random environmental variables

Table G.1 P-values corresponding to symmetric rank correlation matrix among the controlled factors.

	Age	Type	Flooring
Age	--	p=0.865	p=0.729
Type	--	--	P=0.842
Flooring	--	--	--

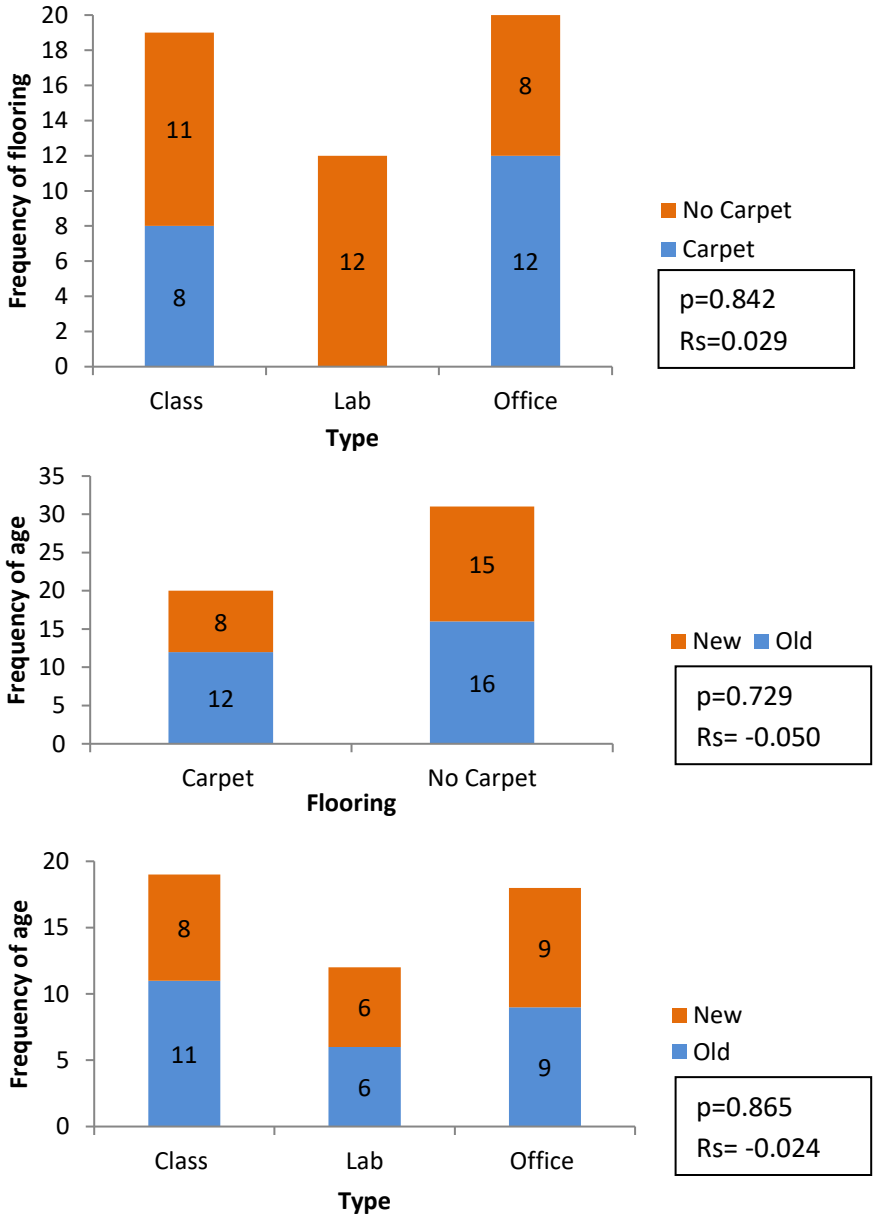
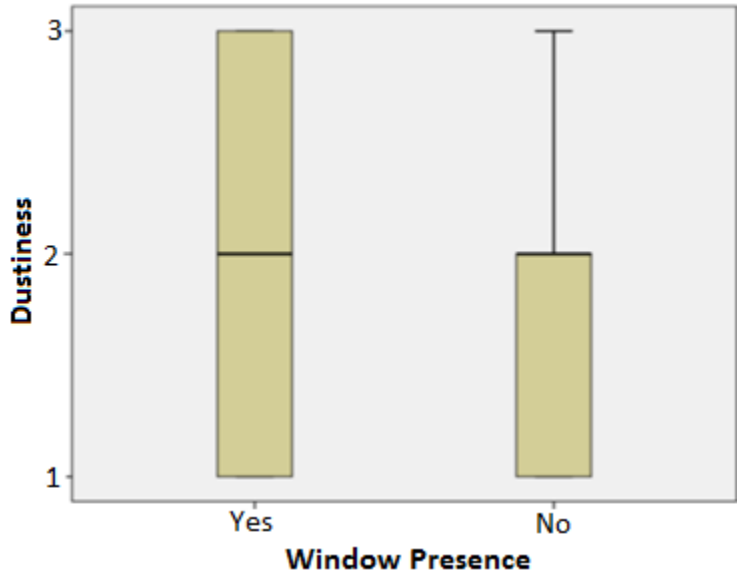


Figure G.1 The correlation between the controlled factors of age, type of room, and flooring.

Table G.2 P-values corresponding to symmetric rank correlation matrix among the uncontrolled factors.

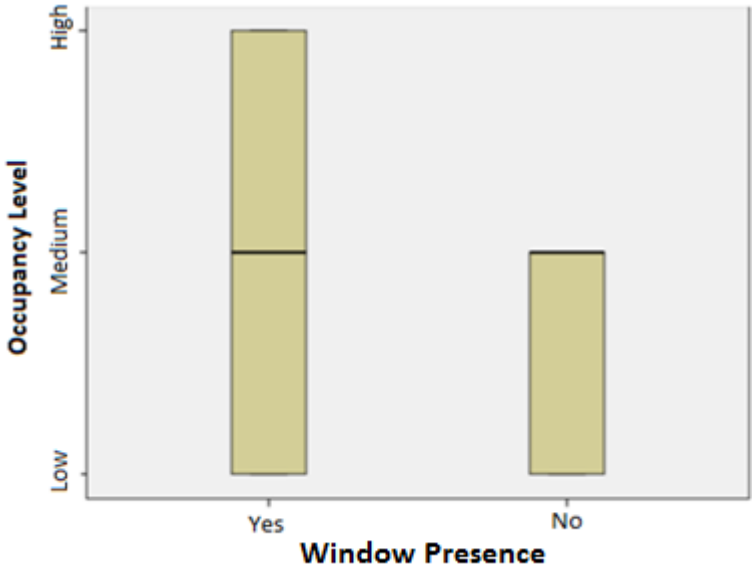
	Window presence	Dustiness	Occupancy level	Temperature	Humidity
Window presence	--	p=0.473	p=0.527	p=0.326	p=0.760
Dustiness	--	--	P=0.021	p=0.002	p=0.007
Occupancy level	--	--	--	p=0.195	p=0.496
Temperature	--	--	--	--	p=0.000
Humidity	--	--	--	--	--

a)



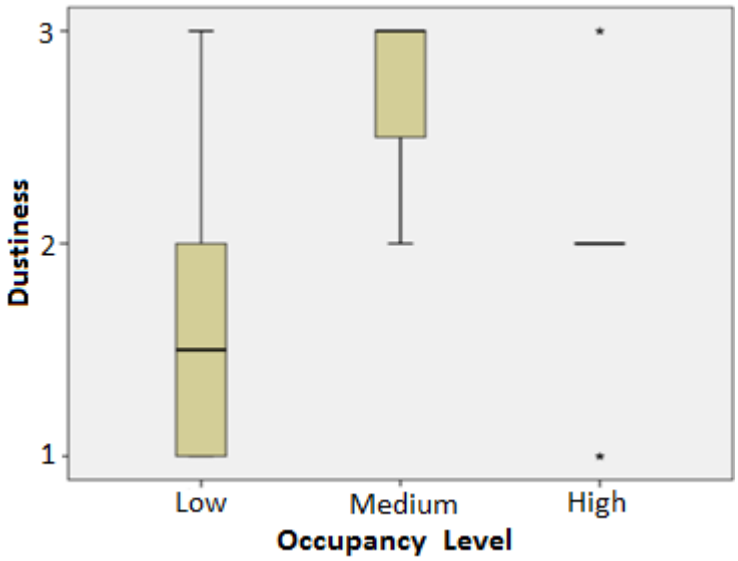
P=0.473
Rs=0.103

b)



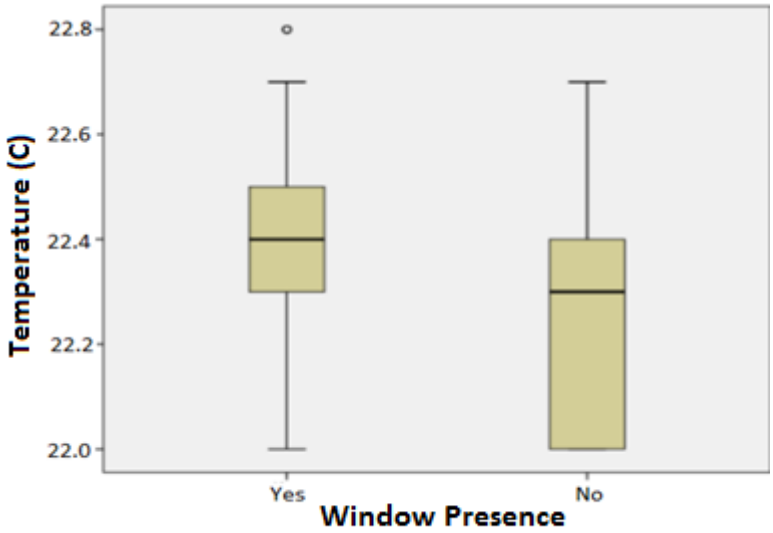
P=0.527
Rs=0.120

c)



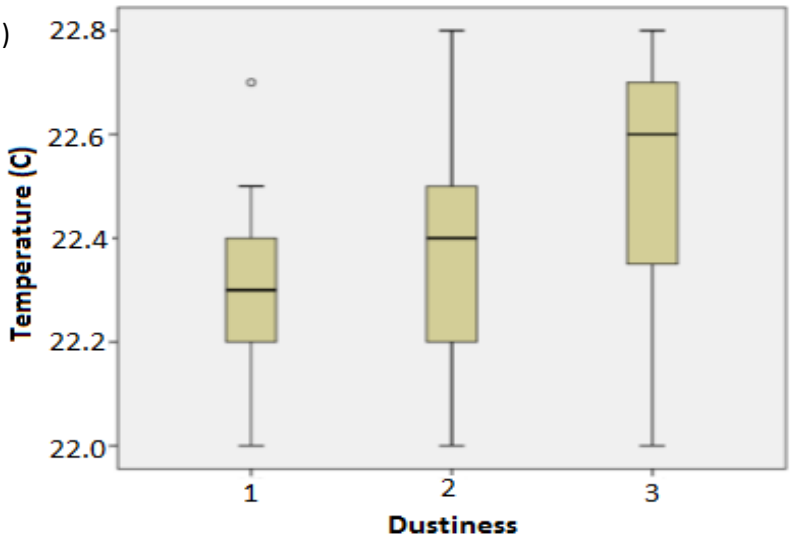
P=0.021
Rhs=0.322

d)



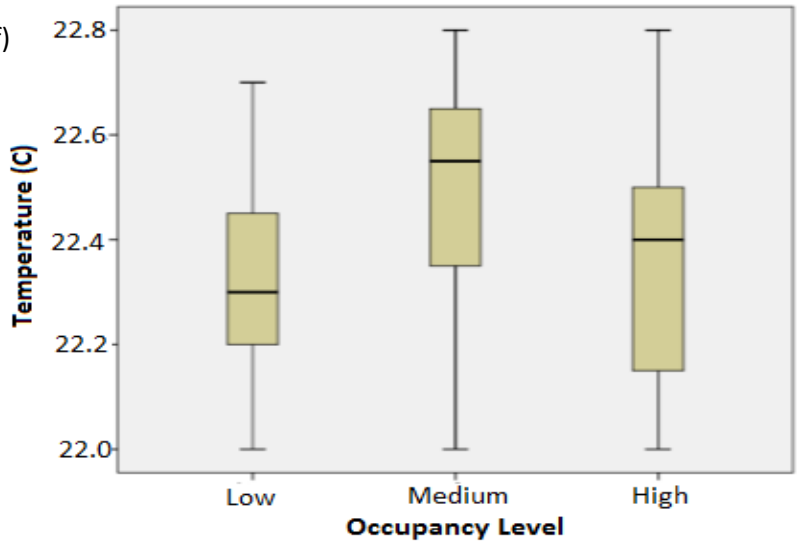
P=0.326,
Rs=0.146

e)



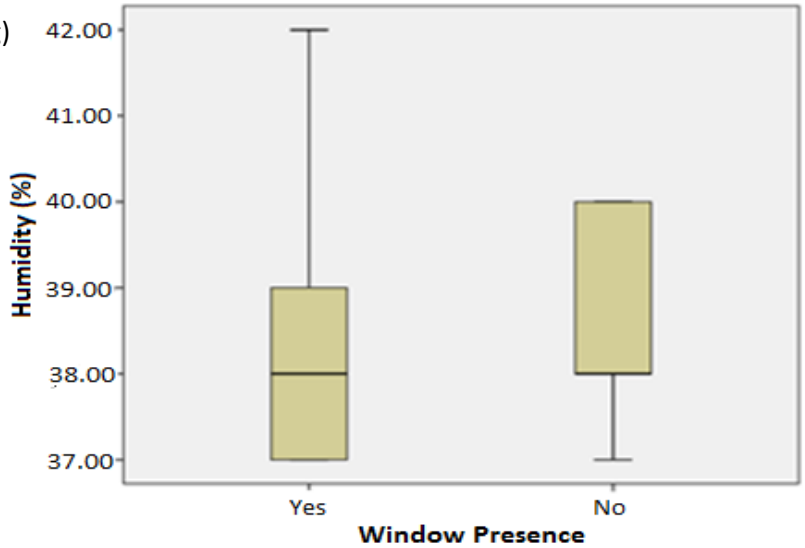
P=0.002,
Rs=0.420

f)



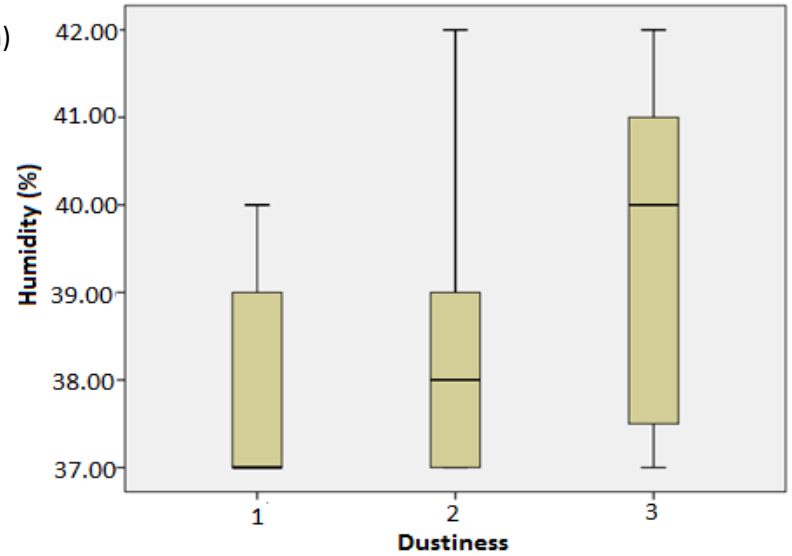
P=0.195,
Rs=0.185

g)



P=0.760,
Rs=-0.044

h)



P=0.007,
Rs=0.372

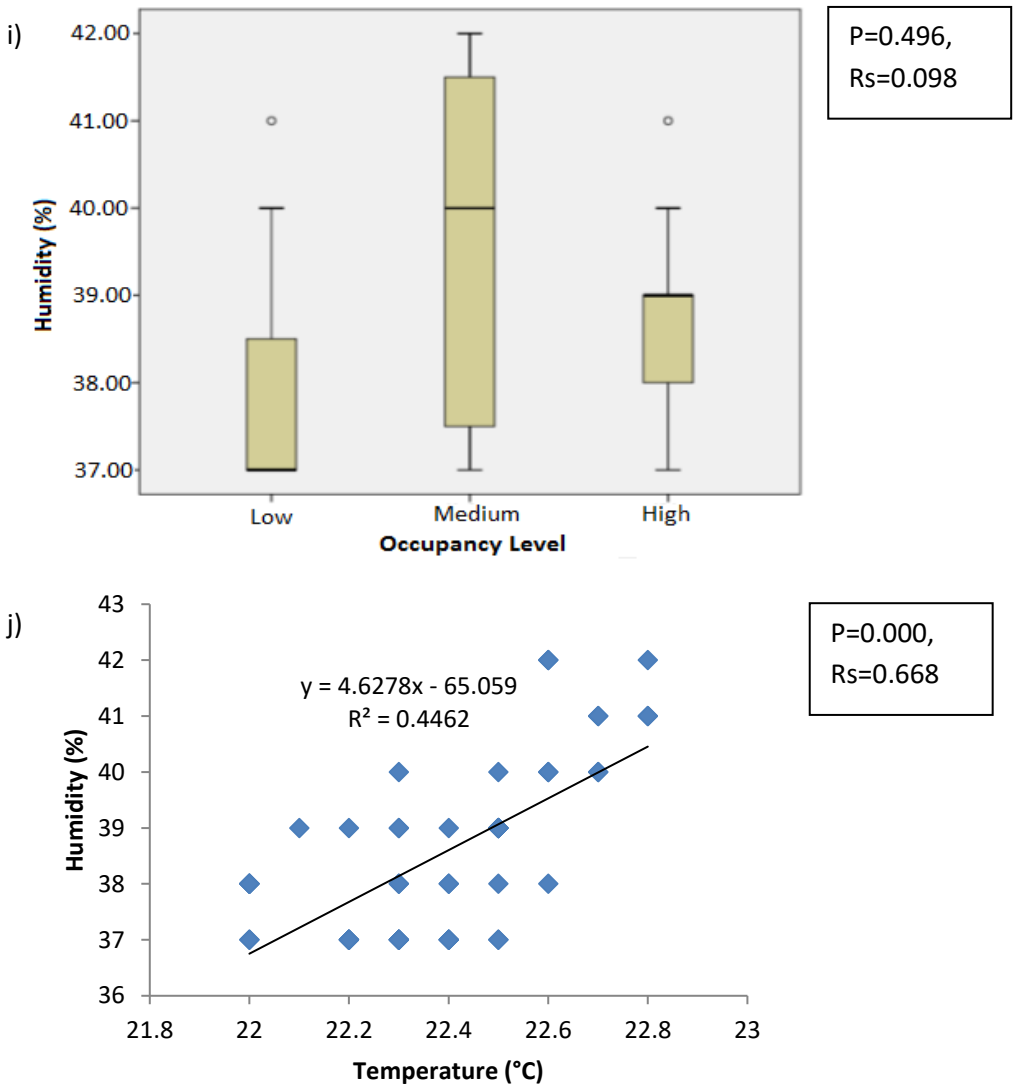


Figure G.2 The uncontrolled factors of temperature, humidity, occupancy level, dustiness, and window presence and their correlations.

Appendix H- Supplementary table and figure for the correlation between weight loss and tensile strength

Table H.1 The correlation analysis between weight loss and stress of drywall samples.

			Weight	Stress
	Weight	**Pearson Correlation**	1	-.924**
		Sig. (2-tailed)		.003
		N	7	7
	Stress	**Pearson Correlation**	-.924**	1
		Sig. (2-tailed)	.003	
		N	7	7

** Correlation significant at the 0.01 level (2-tailed)

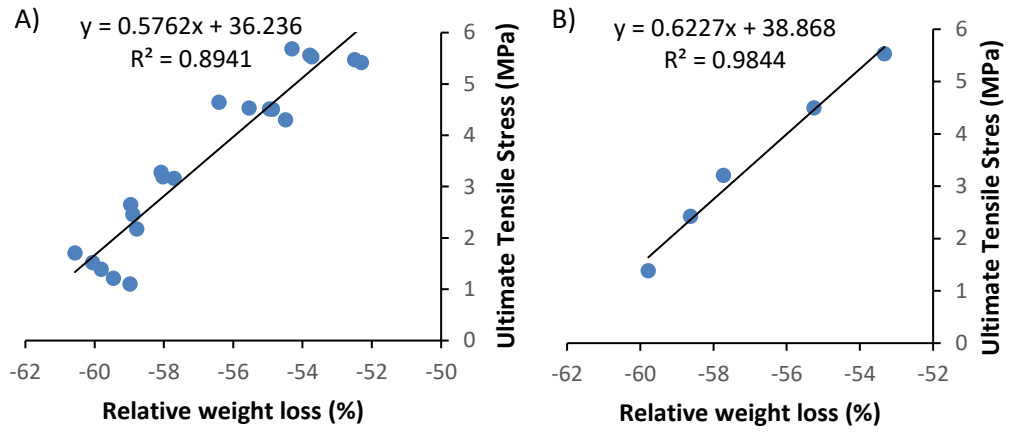
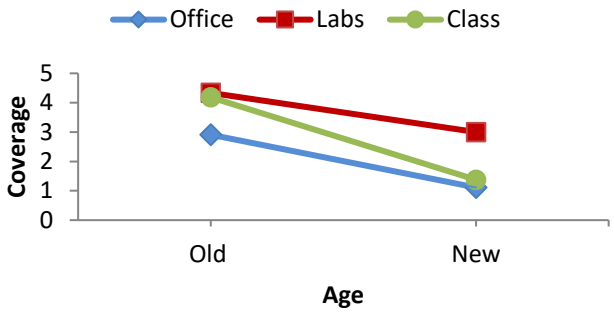
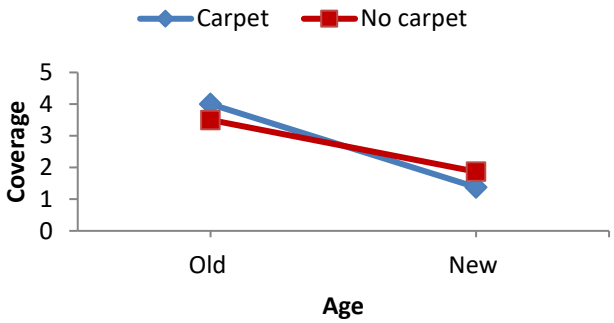


Figure H.1 High correlation between weight loss and maximum stress of drywall samples a) for all 51 rooms, and b) the average of all 51 rooms.

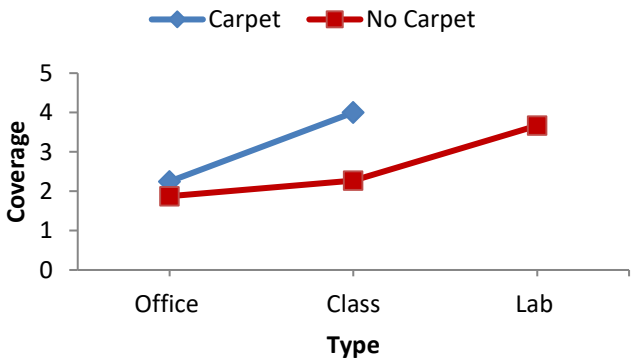
Appendix I- Supplementary figures for the interaction of the three controlled factors tested for coverage and diversity



(a) Age of buildings and types of room interaction for fungal coverage on drywall samples

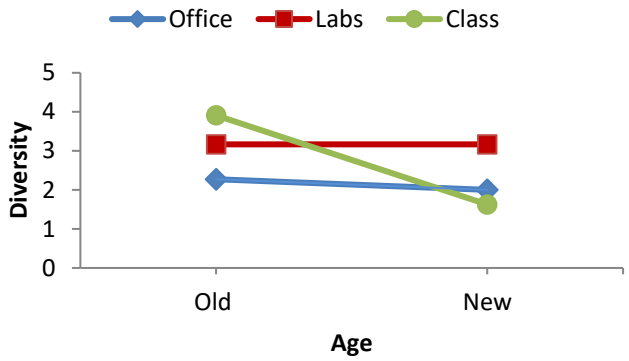


(b) Age of buildings and type of flooring interaction for fungal coverage on drywall samples

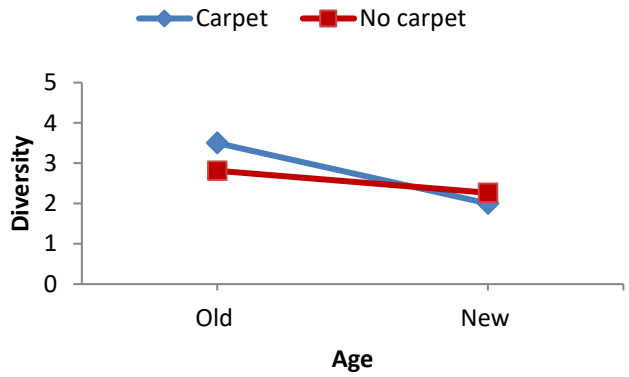


(c) Types of room and type of flooring interaction for fungal coverage on drywall samples

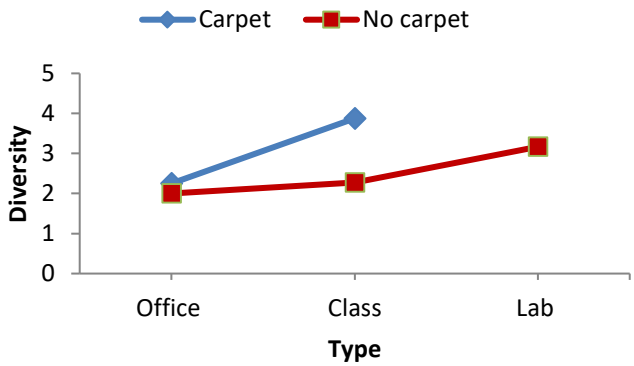
Figure I.1 The interaction between the three controlled factors tested for fungal coverage on drywall samples.



a) Age of buildings and types of room interaction for fungal diversity on drywall samples



(b) Age of buildings and type of flooring interaction for fungal diversity on drywall samples



(c) Types of room and type of flooring interaction for fungal diversity on drywall samples

Figure I.2 The interaction between the three controlled factors tested for fungal diversity on drywall samples.