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EVALUATION OF EXPOSURE TO AEROSOLS AND BIOAEROSOLS THROUGH ADVANCED TOOLS AND INTERDISCIPLINARY APPROACHES

By

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A dissertation submitted to the

School of Graduate Studies

Rutgers, The State University of New Jersey

In partial fulfillment of the requirements

For the degree of

Doctor of Philosophy

Graduate Program in Environmental Sciences

Written under the direction of

Gediminas Mainelis

And approved by

New Brunswick, New Jersey

MAY, 2020

ABSTRACT OF THE DISSERTATION

Evaluation of Exposure to Aerosols and Bioaerosols Through Advanced Tools and Interdisciplinary Approaches

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Dr. Gediminas Mainelis

Adverse health effects caused by ambient air pollutants are well-documented; those negative effects are further amplified by our exposure to indoor pollutants, especially since we spend about 90% of our time indoors and 70% of that in homes. The goal of this dissertation is to connect the investigation of aerosol and bioaerosol exposures in indoor and outdoor environments with health symptoms and properties of the built environments. Given that multiple factors affect our exposures to aerosols and bioaerosols, this dissertation aims to improve our ability to measure such exposures in both indoor and outdoor environments using advanced portable and personal samplers and then integrate the exposure data with building design features, environmental parameters, and people's health.

Specifically, the aim of the Dissertation is to 1) investigate exposure to **aerosols** using indoor air quality (IAQ) techniques with advanced **portable aerosol samplers** in two residential multi-apartment buildings and then associate the data with questionnaire results and building deficiencies detected using spatially resolved infrared thermography imaging. 2) Assess the presence and seasonal variability of culturable **bioaerosols** using **portable** **samplers** in three multi-apartment residential buildings and associate them with ventilation system types and environmental parameters. 3) Evaluate the relative biological performance of commercially available and recently developed **personal samplers** to determine personal **bioaerosol** exposures in indoor and outdoor environments accurately.

Aim 1: Structural building deficiencies like missing wall insulation, apartment location, and occupant behavior, such as smoking and the use of candles or incense indoors, contributed to the overall presence and accumulation of ultrafine particles (< 300 nm size) indoors. The measured ultrafine particles and PM_{2.5} were associated with resident reports of asthma attacks in the last 12 months. *Aim 2:* Culturable fungi concentrations were lower in buildings with central heating and cooling, while culturable bacteria concentrations did not differ between buildings with window AC and central heating/cooling. Nonetheless, the median values of culturable fungi and bacteria indoor-outdoor (I/O) ratios suggested indoor accumulation of outdoor fungi and prominent indoor sources of bacteria in addition to outdoor sources. Indoor bioaerosol concentrations had a significant and positive association with the indoor dew point. *Aim 3:* The collection mechanisms, collection media, and sample retrieval techniques of the personal samplers likely influenced their ability to measure personal bioaerosol exposure in different environments. The obtained sampler performance data and insights into their use provide information that would be useful for further sampler developments and also when choosing tools for personal bioaerosol exposure assessments.

In conclusion, this research provides an example of a holistic approach for aerosol and bioaerosol exposure assessment that includes evaluation of building design, ventilation systems, occupant health, environmental parameters, and application of different sampling technologies.

ACKNOWLEDGMENT

I want to thank my teachers, family, and friends who believed in me and supported me throughout the last five and a half years in graduate school. First, I would like to sincerely express my profound appreciation and gratitude to my advisor, Dr. Gediminas Mainelis, for his constant support and patience. Dr. Gedi has an open-door policy, which made it very easy for me to meet with him and discuss ideas and results. He is enthusiastic about my research and open to new ideas. He was always available to lend an ear and encouraged me to expand my thoughts. Being an international student, I would not be at this stage without the various funding positions made available to me by him. He has transformed the way I would go about solving scientific questions. He is my mentor, but he is also a friend who has advised me on many topics pertaining to life as a graduate student. I am forever grateful to him for accepting me into his laboratory in January of 2015, and I am proud to be a member of his laboratory.

Second, I would like to sincerely thank my dissertation committee and other professors for the multiple roles they played in my graduate school career. My external committee member, Dr. Clinton J. Andrews, has always been gracious and supportive. Working with him on projects, presentations, and finally, my dissertation has been an enjoyable, encouraging experience. Dr. Donna E. Fennell has always been approachable, friendly, and helpful whenever we met. Her feedback used in shaping my dissertation has been greatly beneficial. I can't express enough my sincerest appreciation to Dr. Taewon T. Han, who I have worked with in the laboratory for the past three and a half years on several projects. He is one of the kindest, understanding researchers that I have had the fortune to learn from and work together. Bouncing ideas back and forth with him has been a very stimulating experience. I would also like to thank Dr. Peter F. Strom and Dr. John R. Reinfelder for serving on my candidacy committee and always greeting me with an enthusiastic smile. I want to thank the graduate program directors of the Environmental Sciences Department (ENR), Dr. Daniel Giemenz (former) and Dr. Lisa A. Rodenburg (current), for nominating me for SGS awards and filling all the paperwork needed in a timely fashion. Dr. Clifford P. Weisel (program chair of the Exposure Science Department) and Dr. Brian T. Buckley have been encouraging as well.

Third, I would like to sincerely thank my current lab members, Leonardo Calderón, Sydonia Manibusan, and Dr. Hyeon-Ju Oh, and my former lab member, Dr. Jennifer H. Therkorn, for making lab interesting and fun. Jen and Leo took me under their wings and taught me the important techniques I needed for my various projects. I will always be grateful to both of them. Syd and Oh have been tremendously supportive and helpful in the past few years. I also want to acknowledge the support from my other friends whom I met at different points of graduate school. The staff of ENR has my heartfelt appreciation for their hard work: Melissa Arnesan, Martha Pineda, Dawn Skouboe, Daniel Manzo, Rita Lehman, Veronica Tompkins, Maria Rivera, and Jeannie Nicewicz.

Fourth, I would like to highlight the two student organizations that provided me with friendships and laughter: RESSA (Rutgers Exposure Science Student Association) and ESGSA (Environmental Sciences Graduate Student Association). I would also like to acknowledge my various award benefactors: poster competition awards from the American Association of Aerosol Research (AAAR) and the North and Central NJ Air and Waste Management Association; scholarship from the New Jersey Water Environment Association; and the travel and professional development funds from Rutgers University. Fifth, I can't adequately thank my parents, Dr. Thomas Joseph and Dr. Shila M. Thomas, enough for always believing in me and giving me more than I could ever imagine. I am here today because of their love, prayers, and sacrifices. Roshini E. (sister), Jose (brother-in-law), and Jake J. (nephew) Kurian have always brought a smile to my face and encouraged me to improve. I also want to thank my future in-laws especially, the Rev. Dr. William C. Myers and Margaret E. Myers, for supporting and praying for me and for understanding the effort needed for this process. K. Elizabeth Myers (my future sibling-in-law) has also continually been there for me when I needed her.

And lastly, but most importantly, I want to thank my fiancé, William E. Myers, for his unconditional love that inspires me to achieve my goals. Will's support every single day has made graduate school seem effortless and feasible. I will forever cherish his support and remember the time we have spent in graduate school together.

DEDICATION

To my parents, Dr. Thomas Joseph and Dr. Shila Thomas

To my fiancé, William Estabrook Myers

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CHAPTER 1. INTRODUCTION

1.1 Background

1.1.1 Airborne particulate matter

Airborne particulate matter (PM) is a complex mixture of solid particles and liquid droplets suspended in the air (Agranovski, 2010; Cox & Wathes, 1995). These aerosols could be broadly classified by their source, i.e., natural or human-made, and they can be emitted directly as primary aerosols from fires, construction sites, smokestacks, or they can be formed as secondary aerosols as a result of gaseous reactions involving precursor emissions (US EPA). The size, shape, chemical and biological contents of aerosols characterizes the role they play in the ecosystem and to human health (Agranovski, 2010).

Bioaerosols are a part of PM that has a biological origin, such as viruses, bacteria, fungal spores, fragments of fungal mycelium, pollens, and their by-products (toxins) (Cox & Wathes, 1995; Fröhlich-Nowoisky et al., 2016). Bioaerosols enter the atmosphere due to their release and dispersion from composting facilities, bodies of water, urban environments, pets, humans, heating, ventilation, and air-conditioning (HVAC) systems, soil, and other sources (Burge, 1995; Cox & Wathes, 1995). The ability of bioaerosols to act as cloud condensation nuclei (CCN) or ice nuclei (IN) could also affect the hydrological cycle and precipitation patterns (Fröhlich-Nowoisky et al., 2016).

The ubiquitous presence of aerosols and bioaerosols and their varying spatial and temporal signatures makes it important for researchers to pinpoint their composition and effects on ecosystems and human health (Yoo et al., 2017).

1.1.2 Negative Health Effects of Aerosols and Bioaerosols

Exposure to aerosols and bioaerosols is known to cause negative health effects such as early mortality (Klemm & Mason, 2000; Schwartz et al., 1996; US EPA, 2014; Q. Wang et al., 2018), exacerbation of respiratory tract diseases such as hypersensitivity pneumonitis (Falkinham, 2003), asthma and chronic obstructive pulmonary disease (COPD), reduced lung function (Douwes et al., 2003; Hellebust et al., 2018; Humbal et al., 2018; Srikanth et al., 2008; Xu et al., 2018), cardiovascular diseases (Dabass et al., 2018; Dominici et al., 2006; Erqou et al., 2018), mucous irritation and infections in immunocompromised persons and general population (Hansen et al., 2012; Pankhurst et al., 2011; Tageldin et al., 2017). *Cladosporium, Penicillium, Stachybotrys spp.*, and *Aspergillus* fungal genera are common fungal species causing allergic responses and infections (Garaga et al., 2019; Karimpour Roshan et al., 2019). Children's respiratory and immune systems are vulnerable to bioaerosol exposure even before birth (Sly & Bush, 2019).

The PM in outdoor air pollution is also classified as carcinogenic to humans (IARC Group 1) by the International Agency for Research on Cancer (IARC) (Loomis et al., 2014). Every 10 μ g/m³ increase in PM_{2.5} particle concentration (particles <2.5 μ m in diameter) results in a 6% to 18% increased risk of cardiopulmonary disease and increased all-cause mortality (Eftim et al., 2008; Pope et al., 2002, 2004); every 10 μ g/m³ increase in PM₁₀ particle concentration (particles <10 μ m in diameter) is associated with 0.2 % to 0.6% increase in all-cause mortality (Janssen et al., 2013; Samoli et al., 2008).

1.1.3 Indoor Aerosol Exposure

Negative health effects of ambient air pollutants are further amplified when combined with high levels of indoor pollutants, mainly because people spend about 90% of their time

indoors (Klepeis et al., 2001). Studies have investigated indoor aerosol exposures due to indoor sources including tobacco smoking (Nazaroff & Singer, 2004; Petrick et al., 2011), e-cigarettes (Wills et al., 2019), cooking (See & Balasubramanian, 2006), candles and incense burning (Manoukian et al., 2013), cleaning (Nazaroff & Weschler, 2004; Thatcher & Layton, 1995), secondary organic aerosols (Ji & Zhao, 2015), 3D and laser printers (Chan et al., 2018; Hänninen et al., 2010) and resuspension of deposited particles (Qian et al., 2008, 2014; Sagona et al., 2015; Shalat et al., 2011; Thatcher & Layton, 1995). These sources and the resulting pollutants affect indoor air quality (IAQ), and, in turn, occupants' health (Brussee et al., 2005; Butz et al., 2011; Chalupa et al., 2004; Delfino et al., 2004; Garaga et al., 2019; Platts-Mills, 1994; Tong et al., 2018).

1.1.4 Indoor Bioaerosol Exposure

Due to adverse health effects on workers due occupational exposure to bioaerosols (Pearson et al., 2015), indoor bioaerosol exposures have been researched extensively in occupational settings, including composting facilities (Bünger et al., 2000; Domingo & Nadal, 2009), agriculture, livestock, and food production (Fischer & Dott, 2003; S.-A. Lee et al., 2005), and the waste recycling industry (Lavoie et al., 2006; Marchand et al., 1995; Poulsen et al., 1995). Non-occupational environments such as offices (Hsu et al., 2012; Zhu et al., 2003), hospitals (Lai et al., 2014; Nourmoradi et al., 2012), and schools (Godwin & Batterman, 2007; Hussin et al., 2011; Nevalainen et al., 1991) have also been investigated worldwide. As a result of these studies, associations of wheezing, chronic cough, and subjective respiratory symptoms with damp/moldy surroundings have been well-established (Fung & Hughson, 2003). Bioaerosol concentrations indoors can reach up to ten-fold higher compared to outdoor concentrations (Prussin & Marr, 2015).

Since people spend approximately about 90% of their time indoors and 70% in residences (de Kluizenaar et al., 2017), bioaerosol exposures in single residential homes have also been studied (DeKoster & Thorne, 1995; Fabian et al., 2005; T. Lee et al., 2006). In international studies, culturable bacteria and fungi have been reported by Moon et al. (2014) and Lee & Jo (2006) in high-rise apartment buildings in S. Korea. Apartments, as well as public spaces, have also been investigated for indoor and outdoor bioaerosols in Turkey (Mentese et al., 2012). However, to best of our knowledge, few detailed studies of bioaerosol exposure in multi-apartment residential buildings in the United States (US) are available, even though more than a quarter of the US population lived in apartments in the year 2017 (Statista, 2019).

1.1.5 Building Characteristics and IAQ

In addition to indoor aerosol and bioaerosol sources, building design and features, and their maintenance themselves might affect IAQ (Chenari et al., 2016; Niu, 2004). Asthma and allergies, infections, and sick building syndrome (SBS) have been associated with improper design and maintenance of HVAC systems (Bernstein et al., 2008; Platts-Mills, 1994; Wargocki et al., 2002). Since we spend the majority of our time in homes (de Kluizenaar et al., 2017), it is essential to investigate the state and performance of buildings and how they affect IAQ. Walkthroughs and visual inspections are common procedures used to investigate building conditions and potential deficiencies; however, they are expensive, time-consuming and might miss important building performance characteristics (Balaras & Argiriou, 2002). The development and application of 3D thermal profiles by infrared thermography (IRT) allow a non-destructive, minimally intrusive, accurate, and rapid detection of subsurface deficiencies caused by moisture intrusion and poor construction

quality, making this method superior to visual inspections (Kylili et al., 2014; Meola & Carlomagno, 2004).

IRT can detect heat losses or gains through the building envelope and provide detailed information on building defects and anomalies such as missing, damaged or improperly installed thermal insulation (wet and dry), thermal bridging, and air leakages (Balaras & Argiriou, 2002; Barreira & de Freitas, 2007; Fokaides & Kalogirou, 2011; Kirimtat & Krejcar, 2018; Kylili et al., 2014). In IRT investigations, missing insulation appears as light/dark areas with distinct edges outlining the non-insulated area. A thermal bridge is an area with a higher thermal conductivity than the surrounding surface, and it appears as light/dark areas with linear features as they are often caused by structural components of the building that penetrate the insulation (Balaras & Argiriou, 2002; Craveiro et al., 2018; Guo, 2015). Thermal bridges are also formed due to discontinuities or gaps in the insulation material (Gorse & Johnston, 2012). Observed cracks in building walls can lead to air infiltration or exfiltration (Balaras & Argiriou, 2002), thus affecting the movement of pollutants across the building walls and the presence of pollutants inside the building. However, structural building deficiencies and their impact on IAQ and as well as their relationship with negative health effects are less well understood.

1.1.6 Area and Personal Sampling of Aerosols and Bioaerosols

The two sampling approaches used to determine exposures to aerosols and bioaerosols in indoor and outdoor environments are area and personal sampling. Area sampling is performed with equipment that can often be left unattended for the required sampling time (Quinlan & Plog, 2012). Stationary and portable samplers are used for area sampling to determine exposure levels in our surrounding environments, locate sources of exposure,

and evaluate the effectiveness of any exposure control measures (Leidel et al., 1977; Mainelis, 2019). However, one of the main disadvantages of area sampling is the under- or overestimation of personal exposure (Mainelis, 2019; Sagona et al., 2015; Z. Wang et al., 2012). Personal sampling provides a representative sample of the inhalation exposure of an individual (Haig et al., 2016; T. T. Han et al., 2018; Meadow et al., 2015). Therefore, there is a need to focus on both area and personal sampling.

Over the last two decades, personal exposures to bioaerosols have been characterized using impingers (Duchaine et al., 2001; Zheng & Yao, 2017), rotating cups (Görner et al., 2006), filters (Aizenberg et al., 2000), micro-centrifuge tubes (Su et al., 2012) and recently, electrostatic-based collection (T. Han & Mainelis, 2008; T. T. Han et al., 2017, 2018). However, comparative studies have typically focused on the physical collection efficiencies of the personal samplers and less on their biological collection efficiencies. The biological collection efficiencies measure the losses in viability and culturability of bioaerosols caused by cell damage or stress during sampling (Whyte et al., 2007). The information on the relative performance of personal samplers to capture biological particles in the airborne state—as well as characterization of particles in terms of size distribution, species, viable *versus* dead status, culturable *versus* non-culturable status —are presently limited. Chapters 2 and 3 of this dissertation focuses on area sampling of indoor aerosols and bioaerosols, and chapter 4 focuses on the personal sampling of bioaerosols in indoor and outdoor environments.

1.2 Dissertation Motivation

There is an inadequate understanding of the relationships between exposures to indoor pollutants, health symptoms, and buildings themselves, including their design, structural

anomalies, and maintenance practices (Allacci MS, 2005; Mendell et al., 2002). The fluctuating spatiotemporal signatures of bioaerosols and their negative health effects make it vital to monitor and characterize them, including their size distribution, species, viability, and culturability status. Field studies with stationary and personal samplers can support researchers to gain a more holistic view of IAQ problems in actual buildings. Hence, there is a need to improve our ability to assess exposures to aerosols and bioaerosols in both indoor and outdoor environments using advanced tools and integrate our data with building characteristics, occupant health, and environmental parameters.

1.3 Dissertation Goal and Specific Aims

The *overall goal of this dissertation* is to **evaluate exposure to aerosols and bioaerosols** in indoor and outdoor environments and investigate variables affecting the exposure and the accuracy of its assessment. It has three specific aims.

- Investigate the potential to use, integrate, and correlate three data streams (i.e., traditional indoor air quality investigation, use of questionnaires, and spatially resolved infrared thermography imaging) during IAQ investigations in residential multi-apartment buildings and then use the integrated data to investigate building deficiencies and their role in indoor air quality and residents' health
- 2. Investigate the quantitative and seasonal variability of culturable bacteria and fungi in three multi-apartment buildings in the Northeastern US, taking into account the effect of seasons and building characteristics. The bioaerosol concentrations in multi-apartment residential buildings located in the Northeastern US have not been reported extensively.

3. Evaluate personal exposures to bioaerosols in different environments using five different personal samplers. The relative biological performance of personal samplers in environments with different bioaerosol sources and meteorological factors has not been investigated previously.

1.4 Dissertation Overview

The research presented in this dissertation focuses on the evaluation of exposures to aerosols and bioaerosols through a holistic approach by looking at how the local environments, building characteristics and their deficiencies, and environmental conditions affect exposures.

Chapter 1 describes the motivation for this research and the current need for an integrated approach facing the aerosol and bioaerosol sampling field. The building deficiencies detected by infrared thermography are outlined. Area and personal sampling for exposure assessment using portable and personal samplers are described. The five personal samplers chosen to characterize personal bioaerosol exposure at three distinct sites are described.

Chapter 2 investigates the potential to use, integrate, and correlate three data streams, i.e., traditional indoor air quality investigation, use of questionnaires, and spatially resolved infrared thermography imaging during IAQ investigations in residential multi-apartment buildings. Chapter 2 presents the use of integrated data to investigate building deficiencies and analyze their role in indoor air quality and residents' health.

Chapter 3 investigates the presence and seasonal variability of airborne bacteria and fungi concentrations in three multi-apartment residential buildings and examines building factors and environmental parameters affecting bioaerosol presence. Chapter 3 investigates

the variability of indoor bioaerosol concentrations as a function of ventilation system type, i.e., the presence of central heating, ventilation, and air conditioning systems (HVAC), or hot water baseboard heating with natural ventilation and window air conditioners (AC). Additionally, this chapter describes the association between indoor culturable bioaerosols and indoor DP levels.

Chapter 4 evaluates the relative biological performance of five different personal samplers when evaluating personal bioaerosol exposures in different environments. This chapter examines how the choice of the personal sampler can affect the determined exposures to fungi and bacteria. The biological performance of the personal samplers is assessed in terms of total bioaerosol number concentrations, ATP concentrations, retrieved proportions of live and dead cells, and culturable concentrations.

The summary, implications, and future directions are presented in Chapter 5. Suggested holistic approaches for aerosol and bioaerosol exposure assessments using portable and personal samplers with the inclusion of building characteristics and environmental parameters are presented. Examples of the potential applications of Personal Electrostatic Bioaerosol Sampler (PEBS) active sampler are included. Practical implications and future directions for research are described to advance aerosol and bioaerosol exposure assessments.

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CHAPTER 2. INVESTIGATION OF INDOOR AIR QUALITY DETERMINANTS IN

A FIELD STUDY USING THREE DIFFERENT DATA STREAMS

Material in this chapter has been previously published as:

Nirmala M. Thomas, Leonardo Calderón, Jennifer Senick, MaryAnn Sorensen-Allacci, Deborah Plotnik, Mengyang Guo, Yi Yu, Jie Gong, Clinton J. Andrews, and Gediminas Mainelis. *Building and Environment* 154, (2019), 281 - 295.

2.1 Abstract

Indoor air quality (IAQ) is determined by indoor and outdoor sources and conditions, building characteristics, and occupant behavior. In the field study context where the researcher lacks full control of observational conditions, it is difficult to compare and integrate these determinants because they require such different types and sources of data. This pilot-level project investigated the potential to overcome these limitations by integrating traditional IAQ measurement techniques with the use of questionnaires and analysis of building deficiencies using 3D infrared thermography imaging in two residential multi-apartment buildings. Of the building deficiencies detected by the 3D thermography, missing insulation correlated best with the IAQ measurements and questionnaire data. Apartments missing more than 5% of insulation in their exterior wall (n=6) had a significantly higher number concentration of ultrafine airborne particles (diameter < 300 nm) (p=0.013) and their indoor/outdoor ratio (p=0.029) compared to apartments where less than 5% of insulation was missing (n = 14). The correlation was driven by apartments where no smoking or use candles or incense was reported. Ultrafine particle concentrations in apartments with combustion sources were higher regardless of the levels of missing insulation. Corner apartments had a higher fraction of missing

insulation compared to non-corner apartments (p=0.002); higher levels of missing insulation were detected in apartments where a resident had an asthma attack in the past 12 months. Our data suggest that integration of different data streams produces a more informative IAQ investigation. This pilot-level study should be performed on a larger scale to examine its wider applicability in the IAQ field.

Keywords

- Insulation - Indoor air quality - Ultrafine particles - Building deficiency - 3D thermography - Occupant behavior

2.2 Introduction

Over the past 20 years, studies have shown strong correlations between exposure to ambient particulate matter (PM) and a range of negative health effects, including early mortality (Klemm & Mason, 2000; Schwartz et al., 1996; US EPA, 2014a, 2016; Zhaojun Wang et al., 2018), exacerbation of respiratory tract disease, reduced lung function (Xu et al., 2018), and cardiovascular disease (Dabass et al., 2018; Dominici et al., 2006; Erqou et al., 2018). Every 10 μ g/m³ increase in PM_{2.5} particle concentrations (particles <2.5 μ m in diameter) results in a 6% to 18% increased risk of cardiopulmonary disease and increased all-cause mortality (Eftim et al., 2008; Pope et al., 2002, 2004); every 10 μ g/m³ increase in PM₁₀ particle concentration (particles <10 μ m in diameter) is associated with 0.2 % to 0.6% increase in all-cause mortality (Janssen et al., 2013; Samoli et al., 2008). Furthermore, PM pollutants in ambient air are classified as carcinogenic to humans by the International Agency for Research on Cancer (IARC) (Loomis et al., 2014).

Negative health effects of air pollution are especially pronounced when high levels of outdoor pollution are combined with high levels of indoor pollutants, including PM_{2.5}, PM₁₀, NO₂, SO_x, O₃, and CO (Gouveia & Junger, 2018; Mathieu-Nolf, 2002; William W. Nazaroff & Weschler, 2004; US EPA, 2003). The concern over indoor exposures is amplified by the fact that people spend about 87% of their time indoors (Klepeis et al., 2001). A variety of chemical and biological agents such as environmental tobacco smoke, pesticides, house dust, VOCs, fungi, and allergens, can be present indoors and affect indoor air quality (IAQ), and, in turn, residents' health, including allergic reactions and asthma development and/or exacerbation. The indoor exposure levels to some of these contaminants are thought to have increased with the modernization of housing design, including higher indoor temperatures, extensive use of furnishings and carpeting, improved insulation and weatherization, and low ventilation rates (Ben-David & Waring, 2018; Jia et al., 2008; Kaunelienė et al., 2016; Xiong et al., 2015).

In addition, building design and features themselves might affect IAQ (Chenari et al., 2016; Niu, 2004). However, so far there is inadequate understanding of the relationships between exposures to indoor pollutants, health symptoms, and buildings themselves, including their design, structural anomalies, and maintenance practices (Allacci, 2005; Mendell et al., 2002). While improper design and maintenance of building HVAC systems are known to increase the risk of sick building syndrome (SBS), infections, asthma and allergies (Bernstein et al., 2008; Platts-Mills, 1994; P. Wargocki et al., 2002), structural deficiencies and their relationship to negative health effects are less well understood. Dales et al. suggested that IAQ and its effect on human health in residential buildings were determined by both lifestyle choices (indoor smoking, pets, housekeeping)

and building structure and quality (Dales et al., 2008). Building management including operation and maintenance, and practices are also important determinants of occupant health as they may contribute to IAQ problems (Bonnefoy et al., 2003; Oliver & Shackleton, 1998; Weich et al., 2002).

IAQ depends heavily on context-specific factors including outdoor conditions, building characteristics, indoor sources, and occupant behavior/activities indoors. Laboratory or controlled chamber studies are valuable for carefully documenting specific causal relationships, but field studies are necessary to gain a more integrated view of IAQ problems in real buildings. Walkthroughs and visual inspections are commonly used to investigate building conditions and potential deficiencies; however, they are costly, timeconsuming and, due to their reliance on visual inspection, might miss important building performance characteristics (Balaras & Argiriou, 2002). Typical indoor air investigations rely on air sampling, monitoring, and analysis, which provide information about the quality air that the building occupants breathe, but such methods often are time-consuming and expensive. Structured questionnaires also are used in IAQ studies to obtain information regarding occupants' experiences with the building and its environment, including selfreported health status of household members (Dales et al., 2008; Hansen, 1993; A. J. Lawrence & Khan, 2018; Meng et al., 2005; Zhaojun Wang et al., 2018; Zuocheng Wang et al., 2016; Wong & Huang, 2004; Zhou et al., 2018).

In recent years, there has been an increased use of infrared thermography (IRT), where the resulting 3D thermal profiles of buildings were used to investigate the state and performance of buildings. The development and application of 3D thermal profiles allow a non-destructive, minimally intrusive, accurate, and rapid detection of subsurface

deficiencies caused by moisture intrusion and poor construction quality making this method superior to visual inspections (Kylili et al., 2014; Meola & Carlomagno, 2004). A case study conducted by Ljungberg in 1996 explored the concept of using infrared thermography as an important diagnostic tool to detect sick building syndrome and overview any building related damages (Ljungberg, 1996). An IRT study by Dall'O' et al. sampled 14 existing buildings located in Italy and determined the feasibility of applying this technique to evaluate the energy performance of the buildings (Dall'O' et al., 2013). A recent study assessed the air leakage points in a multi-story residential building in Portugal using an IR camera; it also quantitatively evaluated the potential of using an active IRT in conjunction with an artificial heat source to enhance thermal contrast in defective areas compared to a passive IRT that uses no external excitation energy source (Lerma et al., 2018). With the addition of reliable metrics for quantitative assessment of building performance or quality of building construction, the effectiveness of IRT can be further improved (Guo, 2016). In addition, IRT can be combined with terrestrial laser scanning, i.e., LiDAR (Light Detection and Ranging), to produce a better model resolution and accuracy compared to images obtained in the visible wavelengths, i.e., with an RGB-D camera (Alba et al., 2011) or from an SFM (Structure-from-motion) model (Ham & Golparvar-Fard, 2013).

Overall, IRT can detect heat losses or gains through the building envelope and provide detailed information on building defects and anomalies such as missing, damaged or improperly installed thermal insulation (wet and dry), thermal bridging, and air leakages (Balaras & Argiriou, 2002; Barreira & de Freitas, 2007; Fokaides & Kalogirou, 2011; Kirimtat & Krejcar, 2018; Kylili et al., 2014). In IRT investigations, missing insulation

appears as light/dark areas with distinct edges outlining the non-insulated area. A thermal bridge is an area with higher thermal conductivity than the surrounding, and it appears as light/dark areas with linear features as they are often caused by structural components of the building that penetrate the insulation (Balaras & Argiriou, 2002; Craveiro et al., 2018; Guo, 2015). Thermal bridges are also formed due to discontinuities or gaps in the insulation material (Gorse & Johnston, 2012). Observed cracks in building walls can lead to air infiltration or exfiltration (Balaras & Argiriou, 2002), thus affecting the movement of pollutants across the building walls and the presence of pollutants within the building. All of these deficiencies have potential effects on IAQ. Several publications have shown a reduction in sick building syndrome (SBS) symptoms in indoor environments with increased ventilation rates (Fisk et al., 2009; Seppänen et al., 2006; Sundell et al., 2011), including improved schoolwork performance with increased outdoor air supply (Pawel Wargocki & Wyon, 2013). However, there could be an increase in indoor particle concentration and ozone exposure in heavily polluted areas with higher outdoor air supply rates (Carrer et al., 2015). Hence, the observed missing insulation and its contribution to passive ventilation rate might be beneficial or detrimental depending on outdoor air quality and conditions.

All these mentioned building and indoor air investigation techniques, i.e., indoor air quality investigation, questionnaires, and spatially resolved infrared thermography imaging provide valuable insights into IAQ and building conditions, but typically are used separately, especially when it comes to infrared thermography or its combination with terrestrial laser scanning. We suggest that the integration of terrestrial laser scanning and infrared thermography with traditional air sampling and questionnaire usage in IAQ studies brings new opportunities for identifying and diagnosing various housing-related health and IAQ issues. However, the use of such an integrated approach to detect housing-related health issues has not yet been explored.

Thus, the main goal of this pilot-level project was to investigate the potential to use, integrate, and correlate three data streams (i.e., traditional indoor air quality investigation, use of questionnaires and spatially resolved infrared thermography imaging) during IAQ investigation in residential multi-apartment buildings and then use the integrated data to investigate building deficiencies and their role in indoor air quality and residents' health.

2.3 Methodology

2.3.1 Study Sites

The study was designed as an evaluation of building attributes, including potential deficiencies, indoor air quality, and residents' perceptions about their health and building conditions. We partnered with WHEDco (Women's Housing and Economic Development Corporation; Bronx, NY) to assess two high-rise affordable housing buildings they own: Building 1 located in the Bronx, New York, and Building 2 located in South Bronx, New York. Both buildings house low-income and otherwise vulnerable populations who are predominantly African American, Hispanic or Latino. Building 1 was built in the 1920s, retrofitted in 2006 and contains 132 apartments. Building 2 is an EPA Energy Star certified building that was built in 2009 and contains 128 apartments. A more detailed description of Building 2 is provided elsewhere (Jordán-Cuebas et al., 2018). The terms "apartment" and "household" are used interchangeably. Building 1 has "masonry walls with brick façade that were insulated with fiberglass batts during the 2006 renovations. The interior walls are of gypsum and plaster with an air gap" (*Retro Commissioning Report by*

Greenwich Energy Solutions provided to WHEDco, 2013). Building 2 has "exterior walls made out of brick and CMU block construction with 2" rigid exterior insulation and 3 ¹/₂" of fiberglass batt insulation on the interior" (Salmon & Gleeson, 2012; Vijayakumar, 2009).

2.3.2 Field Data Collection

In Building 1 (Figure 2-1a), data were collected in four apartments in February 2016. Additional data from 15 apartments in the same building were collected during the summer season of 2014, but they were not used for this study because the temperature difference between indoors and outdoors turned out to be too low for the infrared detection system to detect deficiencies (data not shown). The lack of temperature difference was at least partially due to windows kept predominantly open during summer. In Building 2 (Figure 2-1b), data were collected in 16 apartments during four data collection trips from 3/14/2015 to 3/20/2015. For all 20 apartments, the collected data included infrared images, terrestrial LiDAR data, digital images of the exterior building structure and interior walls of apartments, indoor humidity and temperature, mass and number concentrations of various airborne particulate matter fractions and real-time weather data from a nearby weather station. Questionnaires investigating residents' health and their perception of building conditions were also administered during data collection. A flow chart describing the methodology of the study is shown in Figure 2-4.

2.3.3 Infrared thermography, laser scanning, and sensor data fusion

This study combined terrestrial laser scanning using the FARO Laser Scanner Focus^{3D} (FARO Technologies, Korntal-Münchingen, German) with Light Detection and Ranging (LiDAR) technology, and infrared scanning using the FLIR T650sc camera (FLIR

Systems, 27700 SW Parkway Ave. Wilsonville, OR, USA). The research methodology when using this combination of instruments is explained in detail elsewhere (Guo, 2016). The developed methodology was used to generate a 3D thermal model, and the overall flow diagram of the process is shown in Appendix Figure A-1. Briefly, the steps involved in producing the needed 3D thermographic data were: 1) collection of infrared (IR) data that include both color and temperature information for every point in the image and processing of the image into a data matrix that preserves temperature information; 2) terrestrial laser scanning of the buildings to obtain three dimensional information about the buildings and use that data to generate 3D point clouds; 3) stitching of infrared images (Figure 2-2a) and their temperature-based segmentation (Figure 2-2b) to isolate and pinpoint areas with different temperatures; 4) projection of infrared temperature segmentation results to 3D point clouds (Figure 2-3a); and 5) 3D thermal point cloud segmentation (Figure 2-3b) to detect structural elements and quantify building deficiencies or attributes that are relevant to building performance. The deficiencies are described by their extent (i.e., area in the image), location in the building, and temperature information extracted from the 3D thermal point cloud.

2.3.4 IAQ Measurements

IAQ was measured in 20 participating apartments (4 in Building 1 and 16 in Building 2). For all days when indoor measurements were performed, equivalent measurements were performed outdoors. All used real-time monitors were calibrated by manufacturers prior to our study. Table 2-1 details the measured parameters and the used instruments as well as the instruments technical characteristics; we also provide references that used instruments in a similar way.

Temperature, relative humidity, carbon monoxide, and carbon dioxide were measured and data-logged using an IAQ-Calc Indoor Air Quality Meter 7525 (TSI Inc., Shoreview, MN). Particle size distribution was measured using AeroTrak Handheld Optical Particle Counter 9306 (OPC; TSI, Inc.), which counts particles in five size channels ranging from 0.3 to 10 μ m, and the last channel counts particles from 10 to 25 μ m (AeroTrak Handheld Particle Counter 9306, n.d.). Total particle number concentration was measured using a P-Trak ultrafine particle counter (model 8525, TSI Inc.), which counts all particles from 20 nm to 1 µm in size (*P-Trak Ultrafine Particle Counter 8525*, n.d.). These direct reading instruments were operated for 45-60 min in each apartment following previously published methodology (Frey et al., 2015; Patton et al., 2016; Zuocheng Wang et al., 2016), and average values, as well as other statistics (min and max values, 5th - 95th percentile range), were recorded. In the subsequent text, the number concentration of particles below 300 nm refers to the P-Trak-measured concentration minus the concentration of particles > 300 nm to 1 μ m measured by the OPC; and the particle number concentration above 300 nm refers to the total particles measured by an OPC. The combined use of instruments made it possible to estimate number concentrations below 300 nm, and a similar concept has been published elsewhere (Langer et al., 2008). While many studies define ultrafine particles as particles < 100 nm (Martins et al., 2010; Mendes et al., 2018; Penttinen et al., 2001; Seigneur, 2009; Tobías et al., 2018), some studies use a broader definition and include particles up to 300 nm in diameter (Baldauf et al., 2016; Isaxon et al., 2015; S.-B. Lee et al., 2017). For the purpose of this paper, the term ultrafine particles will refer to particles smaller than 300 nm. It has been reported that particles smaller than 300 nm contribute to over 99% of the total particles number concentration in

urban streets (Kumar et al., 2009, 2014); such particles also penetrate deeply into the human respiratory system and are of health concern (Baldauf et al., 2016).

The mass concentration of airborne particulate matter in each apartment was measured and data-logged using Dustrak DRX Aerosol monitor 8534 (TSI, Inc.) for 45-60 min. This instrument provided real-time measurements of airborne particle concentrations corresponding to PM1, PM2.5, Respirable (PM4), PM10, and total PM size fractions. Twentyfour-hour PM_{2.5} concentrations were measured using an SKC Inc. (Eighty Four, PA, USA) Personal Modular Impactor with 2.5 µm cut size and 2 µm pore size 37 mm PTFE filter (SKC Inc.). The required flow rate of 3 L/min was provided by a calibrated XR5000 pump (SKC Inc.). During sample collection, the impactor was mounted on a tripod, connected to a pump enclosed in a noise reducing protective pouch (SKC Inc.) and left in each sampling location for 24 hrs. The collected particle mass and the corresponding airborne mass concentration were determined by weighing each filter using a microbalance (Mettler Toledo, OH, USA) before and after sampling. Prior to each weighing, the filters were equilibrated in a weighing room at a steady temperature (20-22 $^{\circ}$ C) and relative humidity (40%) for at least 72 hours. In the subsequent text, the airborne mass concentration of PM_{2.5} refers to that measured by the impactor. The mass concentration of particles $> 2.5 \mu m$ was determined based on DRX measurements: total PM measured by DRX minus PM_{2.5} measured by DRX.

2.3.5. Interviews

All residents were invited to participate in our study via communications from management and the interview team using meetings and brochures. A similar recruitment approach was used in our previous study (Patton et al., 2016). We conducted individual interviews with one adult in every apartment. Sampling was conducted from June 2014 through March 2016 in accordance with IRB-approved questionnaires and human subject research protocols (14-327M), and the interviewed adult was present during sampling. A headcount of occupants was not taken during sampling. Other residents in the apartments continued their usual daily activities. The study participants from 20 households were asked about their perception of building quality and comfort, including air quality, concerns related to living conditions, household activities that could impact indoor air quality, and health problems in their family, such as asthma events and other illnesses.

2.3.6 Statistical Analysis

Since the indoor air quality dataset was non-normally distributed, non-parametric tests were performed for the analysis. We considered log-transforming the data for analysis; however log-transforming can make the data more variable and skewed, increase the difficulty in interpretation of the results, and provide statistical results often not relevant for the original, non-transformed data (Feng et al., 2014). To avoid these issues, we performed non-parametric testing. The median, 25^{th} and 75^{th} percentile, minimum and maximum values are presented since all the measured data were not normally distributed. The correlations between independent non-linear variables (missing insulation in terms of square feet and percentage of the area relative to the outer wall area) were obtained through Spearman correlation, rs. Independent ordinal variables were associated by gamma correlation, G. A G value <0.3, 0.3 - 0.6, >0.6 was considered to have weak, moderate and strong associations, respectively (Table 14.2 in Healey 2011). When data were stratified into two or three groups, the difference of the mean between the groups was analyzed by the Mann-Whitney U test and Kruskal- Wallis H tests, respectively. For many IAQ

parameters, ratios of indoor to outdoor values (I/O) were determined. Statistical analysis was performed using IBM SPSS Statistics 24.0, and statistical significance was accepted at p-values <0.05 and borderline significance at p-values <0.1.

2.4. Results and Discussion

2.4.1 Infrared Thermography

A total of 1609 infrared images were captured for 20 apartments in two buildings insulated with fiberglass batt. These infrared images were integrated with LiDAR data to generate 3D thermography data. The integrated data were used to locate and identify building deficiencies using the 3D thermal model. Table A-1 in SI illustrates building defects that were detected and quantified. Initial statistical evaluation was performed between all the investigated building parameters shown in Table A-1, IAQ observations and questionnaire data. Among the investigated variables, the total number concentration of particles (d > 20 nm to 1 μ m) correlated with the extent of missing exterior wall insulation with borderline significance (p = 0.069 for the area in ft² and p = 0.098 for %). Hence, we focused on missing insulation and its association with IAQ parameters and residents' health.

Missing thermal insulation was detected using IR cameras as a patch with welldefined edges (Balaras & Argiriou, 2002) (Figure 2-5). The RESNET (Residential Energy Services Network) Interim Guidelines for Thermographic Inspections of Buildings provides standards for the use of IRT in residential and light commercial buildings including information on identification of building deficiencies (RESNET, 2012). According to the RESNET standards and FLIR thermal imaging guidebook (FLIR System, 2011), a minimum temperature difference of 11°C (18°F) between the external and internal surfaces is required during inspection to obtain sufficient information about missing or poor insulation. Once all the missing and poor insulation areas were detected and located, a 3D thermal point cloud was used to determine the physical size of each deficiency. For each apartment, the missing insulation was measured in square feet as well as a fraction (or percentage) of the exterior wall. The two variables had a high correlation: r_s = 0.992, p <0.001. The Insulation Grading Standard designed by RESNET was used to grade the insulation condition of each apartment. The standard classifies the insulation condition into three categories:

Grade I: no deficiencies found using an infrared camera

Grade II: 0.5% to 2% of insulation missing for all inspected walls

Grade III: 2 % to 5% of insulation missing for all inspected walls

In some apartments, the percentage of external wall area with missing insulation was higher than the top range of Grade III. Thus, we added Grade IV to classify the apartments with more than 5% of missing insulation (MI). Given that there were only 20 data points, for further analysis, the apartments were stratified into two groups according to MI % levels: apartments that had less than 5% of MI ("low group") and those that had more than 5% of MI ("high group"), and their summary statistics are presented in Table 2-2. The median MI percentage value for "low" group was 0.77%, and for the "high" group was 9.91%; and the difference was statistically significantly different, according to Mann Whitney U test (U=0.00, p <0.001).

2.4.2 Association of Missing Insulation with Particulate Matter Presence

In order to investigate a possible correlation of missing insulation with ultrafine particle concentration, the particle number concentration was separated into number concentration of particles smaller than 300 nm and the number concentration of particles larger than 300 nm. The descriptive statistics of particle number and mass concentrations are shown in Table 2-3. For each apartment, the ratio of number concentration of ultrafine particles with the total particle number concentration (from than 20 nm to 1 µm) was calculated for each apartment and then averaged across apartments. A similar procedure was applied to PM_{2.5} particles mass concentration. The number concentration of ultrafine particles represented 99.7% of the total particle number concentration (data not shown) (larger than 20 nm to 1 µm) with a median value of $1.50 \times 10^{10} \#/m^3$ ($25^{\text{th}} \%$: $7.31 \times 10^9 \#/m^3$, $75^{\text{th}} \%$: 2.72×10^{10} #/m³), which was similar to studies by Kumar et al. (Kumar et al., 2009, 2014). The indoor/outdoor ratio of ultrafine particle concentrations (<300 nm) ranged from 0.35 to 13.14. The mass concentration of PM_{2.5}, or fine particles, had a median concentration of 28.37 μ g/m³ (25th %: 19.07 μ g/m³, 75th %: 57.61 μ g/m³). The mass concentration of particles $> PM_{2.5}$ represented 50% of the total particle mass concentration (data not shown) measured by the DRX with a median value of 24.57 μ g/m³ (25th %: 16.01 μ g/m³, 75th %: 37.87 μ g/m³), which was similar to studies by Ehrlich et al. and Fromme et al. (Ehrlich et al., 2007; Fromme et al., 2008).

The number concentrations of ultrafine particles in the two apartment groups according to their missing insulation percentage was significantly different as per Mann Whitney U test (U= 15.0, p = 0.013) with a median concentration value of $8.39 \times 10^9 \text{ #/m}^3$ for "low" group and $2.32 \times 10^{10} \text{ #/m}^3$ for "high" group (Figure 2-6a). The particle number concentration for "low" group ranged from 2.64 x 10⁹ to 3.92 x 10¹⁰ #/m³ (25th %: 5.69 x 10^9 #/m^3 ; 75th %: 1.99 x 10^{10} #/m^3), while for the "high" group, the concentration ranged from 1.50 x 10^{10} #/m^3 to 9.86 x 10^{10} #/m^3 (25th %: 1.50 x 10^{10} #/m^3 ; 75th %: 5.73 x 10^{10}

 $\#/m^3$). Kearney et al. reported ultrafine particle number concentration of 2.5 x 10⁹ $\#/m^3$ (median value) in 50 homes during winter, which was lower than the median value of our group with "low" missing insulation (Jill Kearney et al., 2014). The number concentrations of particles larger than 300 nm stratified into the "low" and "high" groups were not significantly different as per Mann Whitney U test (U= 32.0, p = 0.222) (Figure 2-6b). The median values here were similar $(2.32 \times 10^7 \text{ #/m}^3 \text{ for "low" group and } 2.19 \times 10^7 \text{ #/m}^3 \text{ for }$ "high" group). This result suggests that the indoor presence of ultrafine particles is associated with missing insulation, likely due to increased penetration of such particles from outdoors (W. W. Nazaroff, 2004). The observed missing insulation could be due to poor workmanship during its installation or renovation, or due to the settling of insulation. Depending on the proportion of missing wall insulation, a temperature gradient can form between indoor and outdoor spaces leading to tangential air flow, thus creating multiple air entry zones. These zones reduce the thermal resistance of the building and could potentially facilitate particle exchange between outdoors and indoors (Silberstein et al., 1991). While particle measurements in each apartment were performed for only up to 60 min, it appears the time was sufficient to show a positive association between the number concentration of ultrafine particles and missing wall insulation. The association between the missing insulation and the presence of particles was not observed for particles larger than 300 nm, most likely because larger particles have lower penetration efficiency through the building envelope (Liu & Nazaroff, 2001; Mosley et al., 2001). A limitation of this study is the absence of information on outdoor PM sources near the buildings as well as prevailing wind direction and air pressure as that could influence particle penetration. A larger sample size or longer monitoring period was not possible due to the difficulties to schedule

occupants for measurements and interviews, and limited instrument availability for longer measurement times.

When PM_{2.5} indoor mass concentrations were stratified according to missing insulation levels, the difference was not significantly different as per Mann Whitney U test (U = 38.0, p = 0.390) (Figure 2-6c) with median value of 27.48 μ g/m³ for "low" group and 35.87 μ g/m³ for "high" group. The same could be said about the mass concentration of particles larger than 2.5 μ m as per Mann Whitney U test (U = 41.0, p = 0.484) (Figure 2-6d) that showed median values of 26.04 μ g/m³ and 20.94 μ g/m³ for "low" and "high" groups, respectively. Jones et al. observed PM_{2.5} indoor concentrations in the range of 10 to 50 μ g/m³ which was similar to our study (Jones et al., 2000). Since particle mass is proportional to the cube of particle diameter, ultrafine particles do not contribute much to PM_{2.5} mass (Matson, 2005), and the difference in number concentration of particles below 300 nm did not affect the difference in PM_{2.5} concentrations.

We also compared indoor/outdoor (I/O) ratios of number concentrations of ultrafine particles in apartments with different levels of missing insulation. Apartments with higher levels of missing insulation had higher number concentrations of ultrafine particles as well as higher I/O ratios. The median I/O value in the "high" group (50^{th} %: 1.15) was significantly higher than that in the "low" group (50^{th} %: 0.63) (U = 19.0, p = 0.029) (Figure 2-7). Particles generated indoors, such as those produced by occupant behavior, as well as those that penetrate from outdoors contribute to the overall presence and accumulation of particles indoors. Missing wall insulation seems to aid particle penetration from outdoors thus increasing the presence of particles indoors. The median I/O value in the "low" group was below 1, which suggest absence of or limited indoor sources. Jones et al. calculated

I/O ratios to indicate the origin of particles. That paper stated that I/O will be less than or equal to 1 in the absence of or limited indoor sources (Jones et al., 2000). A study by Koponen et al. observed indoor concentrations 10 times lower than outdoor concentrations in an office building near downtown Helsinki and suggested that indoor concentrations were affected by outdoor concentrations with a time lag. The time lag was reduced in half when the ventilation system was switched on (K. Koponen et al., 2001). A study by Kingham et al. monitored the spatial variations of traffic-related pollutants in houses with non-smokers and indicated the median I/O ratio of 0.81 to have contributions from outdoors, mainly from vehicles (Kingham et al., 2000).

If one presumes the same particle generation indoors by both the "low" and the "high" groups, then the I/O difference between the two groups could be attributed to the accumulation of particles that penetrated from outdoors. At the same time, it is known that various indoor combustion processes, e.g., smoking, generate ultrafine particles. Thus, individual residents' behavior could substantially affect the presence of particles indoors. Spengler and Sexton described the increase in particle matter concentrations due to indoor combustion of tobacco and its health implications including to non-smokers and children (Spengler & Sexton, 1983). Therefore, it was important to match the apartments according to their potential to generate ultrafine particles and then investigate the presence of ultrafine particles as a function of missing insulation. Here, apartments were divided into categories depending on the presence or absence of the following indoor activities: smoking, burning of either candles or incense, and smoking or burning of either candles or incense (this category is later referred to as "indoor combustion sources"). The information on residents' indoor activities and age were collected during the interviews; the data are shown in Table

2-4. Here we obtained information on the presence of indoor combustion sources in apartments in general, without acquiring information about specific individuals. Furthermore, the questionnaire asked only about the presence of those combustion sources but not about their strength, e.g., a number of cigarettes smoked or the frequency of smoking. Visual confirmations of these indoor combustion sources were not recorded by the interviewer. The observed airborne concentrations of ultrafine particles in apartments with and without the above-mentioned indoor combustion sources were stratified according to their missing insulation level, and the data are presented in Figure 2-8. For simplicity, the headings in each graph of Figure 2-8 indicate only one combustion source; subsets are discussed in subsequent text and Table 2-4.

In apartments with no smoking (n=14) with a subset of 7 apartments where candles or incense was burnt (Figure 2-8a), the number concentration of ultrafine particles in the "high" group (n = 5 with 3 candles or incense burners) had a median value of 1.87×10^{10} #/m³ (25th %: 1.5×10^{10} #/m³, 75th %: 3.56×10^{10} #/m³), and it was significantly higher than that in the "low" group (n = 9 with a subset of 4 candles or incense burners), which had a median value 7.63 $\times 10^9$ #/m³ (25th %: 5.0×10^9 #/m³, 75th %: 1.28×10^{10} #/m³), with U= 5.0 and p = 0.010. Ultrafine particle number concentration (up to 1 µm) ranging from 2.7 x 10⁹ to 3.7 x 10⁹ particles/m³ (median values) was measured by Kearney et al. in non-smoking homes during the summer and winter seasons, which was lower than the concentration in our "low" group (J. Kearney et al., 2011).

Apartments with no candles or incense burnt (n=8) with a subset of 1 smoker (Figure 2-8b) had a similar relationship of having significantly higher number concentration of ultrafine particles in the "high" group (n = 2 with no smokers; median =

 $3.11 \times 10^{10} \#/m^3$; $25^{\text{th}} \%$: $1.87 \times 10^{10} \#/m^3$, $75^{\text{th}} \%$: $4.36 \times 10^{10} \#/m^3$) than the "low" group $(n = 6 \text{ with a subset of } 1 \text{ smoker; median} = 7.56 \times 10^9 \text{ } \text{\#/m}^3; 25^{\text{th}} \%; 5.55 \times 10^9 \text{ } \text{\#/m}^3, 75^{\text{th}}$ %: 8.85 x $10^9 \#/m^3$) (U= 1.0, p = 0.048). We further looked at apartments with none of the indoor combustion sources (n = 7, Figure 2-8c) and the above-stated relationship persisted and was significantly different. Here, the "low" group (n=5) had a median particle number concentration of 7.2 x $10^9 \#/m^3$ (25^{th} %: 5.0 x $10^9 \#/m^3$, 75^{th} %: 8.39 x $10^9 \#/m^3$) which was higher than that for the "high" group (n = 2) with a median of 3.11 x 10¹⁰ #/m³ (25th %: $1.87 \ge 10^{10} \#/m^3$, 75th %: 4.36 x $10^{10} \#/m^3$) (U= 0.0, p = 0.027). The presence of particles below the size of 300nm in apartments with no combustion or minimal combustion sources was significantly affected. This suggests that in the absence of smoking and/or burning of candles or incense, the number concentration of ultrafine particles was significantly higher in the "high" missing insulation group compared to the "low" missing insulation group, thus increasing personal exposure to ultrafine particles. We speculate that this increase in particle concentration indoors was due to their penetration from outdoors. Diapouli et al. reported the influx of ultrafine particles below 1 µm from outdoor vehicular emissions into classrooms in the absence of indoor sources such as smoking and cleaning. The reported values were comparable to the number concentration of ultrafine particles in the "high" group of this study without or with limited indoor sources (Diapouli et al., 2007).

On the other hand, in the presence of any one of the following particle sources, such as smoking (n = 6 with a subset of 5 candles or incense burners, Figure 2-8d), candles or incense burnt (n = 12 with a subset of 5 smokers, Figure 2-8e) or any two indoor combustion source categories (n = 13, Figure 2-8f), the indoor particle number concentration was not dependent on the levels of missing insulation. This suggests that the production of particles by the combustion sources overwhelmed the influx of ultrafine particles due to missing insulation and, thus, no difference was observed. It is important to point out that occupant behavior played a major role in the observed relationship between the missing insulation and the presence of ultrafine particles. The relationship described above held in apartments with no or relatively low presence of combustion sources, such as smoking and burning of candles or incense. For example, a study by Zhu et al. described the penetration of ultrafine particles (6 - 220 nm) into urban residences located near a freeway. In their study, infiltration of ultrafine particles through the building envelope was reported to be highest for particles in size range of 70 to 100 nm in residences with no known indoor aerosol sources, which is in agreement with our study (Y. Zhu et al., 2005).

On the other hand, in the apartments where such combustion sources were prevalent, the particles generated indoors overwhelmed the contribution of particles from outdoors, and no relationship between the missing insulation and particle presence was observed. Wallace et al. studied ultrafine particles below 10 nm and published a similar result regarding the lower contribution of ultrafine particles from outdoors if indoor sources are present (Wallace et al., 2008).

In order to check the influence of other common indoor particle sources on the presence of particles, the particle presence was analyzed as a function of cooking frequency in each apartment. Based on questionnaire data, 2 residents cooked once a day, 8 residents cooked twice a day, and 10 residents cooked a day thrice. According to Kruskal-Wallis H test, we found that cooking didn't have a significant effect on the number concentration of ultrafine particles (χ^2 (2) = 2.60, p = 0.272) or above 300nm (χ^2 (2) = 3.95, p = 0.139). Interviewees in all apartments were not cooking during the sampling period. Any

contributions to ultrafine particles due to cooking before our interviews have likely dissipated by the time of our monitoring. Nonetheless, the number concentration of ultrafine particles below 300 nm in apartments where combustion sources were present was comparable to the values reported by Isaxon et al. during activities by residents such as using the oven, boiling, toaster, and cleaning $(10^{10} \text{ to } 10^{11} \text{ particles/m}^3)$ (Isaxon et al., 2015). As mentioned above, smoking, burning of incense or candles by the occupants played a major role in particle presence in the apartments. A study conducted in Sweden and Denmark showed an I/O of ultrafine particle number concentration comparable to our study and also demonstrated the increase in particle concentration when there were strong indoor sources such as smoking, candle burning and cooking (Matson, 2005). However, different from that study we did not find an association between the frequency of cooking and the presence of ultrafine particles. Other studies have also shown a significant increase in ultrafine particle presence indoors due to smoking (Alderman & Ingebrethsen, 2011; Valente et al., 2007; Wallace & Ott, 2011) and the burning of candles or incense (Géhin et al., 2008; Vinzents et al., 2005). Even art activities in classrooms showed a similar number concentration of ultrafine particles (below 100 nm) to this study in the presence of combustion sources (Morawska et al., 2009).

We found that 60% of the study's participants had children younger than 14 years and that 67% of smokers had children living with them. The smoking-related particles not only degrade indoor air quality but can also cause developmental issues in children (Klepeis et al., 2017). The residents and their families are exposed to smoking-generated particles not only via first-hand and second-hand smoking but also due to resuspension of deposited smoke-related particles. Resuspension of particles larger than PM_{2.5} from the floor has likely contributed to the presence of such particles in monitored apartments; resuspension of particles has been discussed in our previous studies (Sagona et al., 2015; Shalat et al., 2011). Resuspension of floor-deposited dust would especially elevate exposures of young children due to the proximity of their breathing zones to the floor (Burtscher & Schüepp, 2012).

The resuspension of deposited particles depends on their size (Qian et al., 2014; Qian & Ferro, 2008), while the size of smoking-originated particles depends on the nicotine content and the nicotine delivery device, e.g., cigarette manufacturing technology (Becquemin et al., 2007), e-cigarettes (Glantz & Bareham, 2018), cigars (Baker et al., 2000), or water pipes (Akl et al., 2010). However, our questionnaire did not inquire about the type of smoking, and we cannot speculate about the size of produced particles and how that would affect the exposure of residents and particle lung deposition.

Overall, to advance our understanding of the mechanisms governing the relationship between the missing insulation and presence of particles indoors, a controlled lab study should be conducted where different types of insulation, varied wall thickness and outdoor environmental conditions are considered.

Further analysis was performed to investigate the association between missing insulation and residents' perception of indoor air quality. Residents were asked whether they thought the air in their apartments was dusty. Out of the 15 apartments perceived by the residents as being dusty, 5 apartments had missing insulation above 5%. Association between apartments perceived as being dusty and high levels of MI was moderate, with a G value of 0.333 and, yet not significant (p = 0.543). Reports on sensing any bad odors related to chemicals or garbage from the hallway or inter-apartment spaces were also

recorded in the questionnaire but found to have no association with MI levels (G = -0.286, p = 0.556).

2.4.3 Association between Missing Insulation and Apartment Location

We further investigated whether the apartment location was correlated with missing insulation. Apartments located in corners (7/20 apts.) were strongly and significantly (G= 0.935, p = 0.002) correlated with the high percentage of missing insulation (Figure 2-9a). Specifically, out of six apartments in the "high" group, five were corner apartments. As a consequence, corner apartments had a higher number concentration of ultrafine particles compared to non-corner apartments: the median number concentration of $1.87 \times 10^{10} \text{ #/m}^3$ ($25^{\text{th}} \%$: $1.5 \times 10^{10} \text{ #/m}^3$, $75^{\text{th}} \%$: $3.92 \times 10^{10} \text{ #/m}^3$) for corner apartments and the median number concentration of $7.92 \times 10^9 \text{ #/m}^3$ ($25^{\text{th}} \%$: $5.28 \times 10^9 \text{ #/m}^3$, $75^{\text{th}} \%$: $2.19 \times 10^{10} \text{ #/m}^3$) for non-corner apartments; the difference was statistically significant (U= 17.0, p = 0.012). The number concentration of particles larger than 300nm had a median concentration of $7.49 \times 10^6 \text{ #/m}^3$ ($25^{\text{th}} \%$: $1.95 \times 10^6 \text{ #/m}^3$, $75^{\text{th}} \%$: $4.07 \times 10^7 \text{ #/m}^3$) in corner apartments, and it was lower compared to non-corners apartments with a median number concentration of $2.33 \times 10^7 \text{ #/m}^3$ ($25^{\text{th}} \%$: $1.42 \times 10^7 \text{ #/m}^3$, $75^{\text{th}} \%$: $6.88 \times 10^7 \text{ #/m}^3$); the difference was borderline significant (U= 28.0, p= 0.083).

The number of apartments where residents noticed cracks (n= 10) did not depend on apartment location with respect to corners (4/10 residents noticed cracks in corner apartments vs. 6/10 residents who noticed cracks in non-corner apartments; G= 0.217, p = 0.637). The indoor temperature was marginally lower in corner apartments (mean = 75.17 \pm 4.32 °F; median = 75.5 °F; 25th %: 71.7 °F, 75th %: 78.51 °F) when compared to noncorner apartments (mean = 75.99 \pm 5.41 °F; median = 76.52 °F; 25th %: 74.97 °F, 75th %: 79.28 °F), but not significantly so (U= 38.0, p = 0.276). The apartment R-values, which are indicators of an ability by an indoor space to maintain temperature, were lower in corner apartments (mean = 1.09 ± 0.54 ; median = 0.94; $25^{\text{th}} \%$: 0.85, $75^{\text{th}} \%$: 1.61) compared to non-corner apartments (mean = 1.49 ± 1.21 ; median = 1.21; $25^{\text{th}} \%$: 0.61, $75^{\text{th}} \%$: 2.05), yet not significantly so (U = 36.0, p =0.226). The lower resistance to heat flow could lead to lower insulating properties of the apartments (US DOE, n.d.). Silberstein et al. described how thermal resistance is reduced due to air infiltration through air entry zones, and it is affected by external wind speed and orientation (Silberstein et al., 1991).

Residents in corner apartments were more likely to burn either candles or incense indoors compared to non-corner apartments (G= 0.750, p = 0.047). Seven out of 8 apartments which didn't burn either candles or incense were non-corner apartments. However, the apartments where residents burnt either candles or incense indoors were equally distributed between the corner and non-corner locations at 6 apartments each. Smoking had little or no influence on the ultrafine particle number concentration in corner apartments (G = 0.429, p = 0.374). An equal number of smokers were recorded in both corner (n=3) and non-corner (n=3) apartments. 6/7 of the corner apartments reported indoor combustion sources (G = 0.674, p = 0.105). However, there was also an almost equal number of apartments that reported indoor combustion sources in non-corner apartments (7/13 apts.).

The apartments located on the fifth floor or below (n= 9) had lower amounts of missing insulation (mean = median = 1.29%; $25^{\text{th}} \%$: 0.33%, $75^{\text{th}} \%$: 3.62%) compared to apartments located on floors six and higher (median = 2.31%; $25^{\text{th}} \%$: 0.7%, $75^{\text{th}} \%$:

7.12%); the association was strong yet only borderline significant (G = 0.739, p = 0.062) (Figure 2-9b).

2.4.4 Association of Asthma with Missing Insulation

The Bronx residents are known to have a high prevalence of asthma (DiNapoli, 2014; Karetzky, 1977; Maantay, 2007; Warman et al., 2009), and, therefore, several asthmarelated questions were included in our questionnaire. The summary of the responses is presented in Table 2-5. We investigated several possible associations between the number of asthma cases reported by residents in the past 12 months and our-measured environmental variables. When the number of asthma attacks (n=6) was stratified according to missing insulation levels, the association was positive but not significant (G = 0.111, p = 0.834). The "low" group had 4/6 cases, and the "high" group had 2/6 cases. The missing insulation percentage in apartments where residents did not report asthma attacks in the past 12 months (n=14) ranged from 0.13% to 12.71% (median = 1.53%, 25^{th} %: 0.36%, 75th %: 5.66%), while the missing insulation percentage ranged from 0.55 % to 19.62% (median = 1.57%, $25^{\text{th}}\%$: 0.66%, $75^{\text{th}}\%$: 15.63%) in the apartment where residents reported asthma (Figure 2-10a). The median levels and the minimum values were similar for both groups, but the maximum value was 7% higher on an absolute scale in apartments where asthma attacks were reported.

We further investigated whether there was an association between asthma attacks reported in the last 12 months and PM levels indoors. The number concentration of ultrafine particles was significantly higher in the group with asthma attacks (U= 19.0, p = 0.029), with median number concentration of 2.3 x 10^{10} #/m³ (25th %: 1.07 x 10^{10} #/m³; 75th %: 5.73 x 10^{10} #/m³) for the "Asthma" group compared with the median value of 1.15 x

 10^{10} #/m³ (25th %: 5.69 x 10^9 #/m³; 75th %: 2.05 x 10^{10} #/m³) for the "No Asthma" group (Figure 2-10b). A similar significant association was seen between reported asthma attacks and the number concentration of particles larger than 300 nm (U= 3.0, p = 0.001). Here, the group with reported asthma attacks had in their apartments a median particle number concentration almost 6x higher compared to the non-asthma group: 8.16 x 10^7 #/m³ vs. 1.42 x 10^7 #/m³. The PM_{2.5} levels also showed a significant association with asthma attacks reported in the last 12 months (U= 18.0, p = 0.024) (Figure 2-10c). The median PM_{2.5} mass concentration in apartments with asthma (median = 51.8 µg/m³; 25th %: 34.0 µg/m³, 75th %: 78.26 µg/m³) was twice as high as the median PM_{2.5} concentration in apartments with no reported asthma (median= 23.99 µg/m³; 25th %: 13.51 µg/m³, 75th %: 41.80 µg/m³). Furthermore, occupant behavior, such as smoking indoors (n=6) was also strongly and significantly associated with asthma reports (G= 0.846, p = 0.027, Figure 2-10d).

Out of 14 apartments that did not report asthma attacks, smoking was reported only in 2 apartments, while four residents in 6 apartments with asthma attacks reported smoking indoors; that helps explain higher PM_{2.5} levels in apartments with asthma even though measurements were taken only once. Butz et al. observed PM_{2.5} levels indoors to be twice higher in smoking households than the EPA outdoor standards and related higher PM_{2.5} levels to the higher prevalence of asthma, which was similar to our finding of higher PM_{2.5} concentrations in apartments with asthma cases and smokers (Butz et al., 2011; US EPA, 2014a). Houses without smokers reported mean PM_{2.5} mass concentration of 18 μ g/m³ in a study conducted in England, which is closer to our 25th percentile in apartments with no reported asthma cases and limited combustion sources (Kingham et al., 2000). A study conducted in a classroom measured PM_{2.5} concentrations in the range of 20 μ g/m³, i.e., similar to our group with no reported asthma (Braniš et al., 2005). Additionally, residents in 1 of 6 apartments who reported asthma attacks didn't smoke indoors but had as much as 14.30% of insulation missing. This specific resident also did not report the burning of candles or incense.

The overall prevalence of asthma cases (n=12) was not associated with the number concentration of ultrafine particles (U= 46.0, p = 0.439). However, the number concentration of particles larger than 300 nm was borderline positively associated (U= 29.0, p = 0.072) with the prevalence of asthma: the median concentration twice as high as compared to apartments with no reported asthma: 2.87 x 10^7 #/m³ in apartments with asthma prevalence vs. 1.45 x 10^7 #/m³ in apartments with no reported asthma prevalence. The overall asthma prevalence was not associated with PM_{2.5} mass concentration levels (U = 42.0, p = 0.322). These results differ from the association between the measured environmental variables and the reports of asthma attacks in the past 12 months. The questionnaire did not elicit information about whether the residents developed asthma prior to or during their stay in the investigated homes, making it more difficult to connect asthma prevalence data with our-measured environmental and building variables.

Since the observed concentrations of airborne particles had a significant correlation with the residents' reports of recent asthma episodes, one can conclude that building deficiencies such as missing insulation play a role in residents' well-being. Ultrafine particles are particularly important because of their high surface-to-volume ratio and high potential to absorb toxic air pollutants per unit mass (Delfino et al., 2005; Sioutas et al., 2005; Sultan et al., 2011). A randomized clinical trial study reported the impairment of alveolar gas exchange region and mild small-airways dysfunction in healthy adults when exposed to ultrafine carbon particles (diameter less than 100 nm) (Pietropaoli et al., 2004). Subjects with asthma were reported to have a higher lung fractional deposition during exercising compared to healthy subjects when exposed to ultrafine carbon particles (Chalupa et al., 2004). Penttinen et al. reported an association between particles 10 nm-1 µm and poor respiratory health as measured by self-monitored peak expiratory flow rate in adult nonsmoking asthmatics (Penttinen et al., 2001). A recent study conducted with nonsmoking asthmatics showed an increase in acute systemic inflammation following exposure to airport-related ultrafine particles (Habre et al., 2018). A cross-sectional study of 655 children attending an elementary school in Australia showed a positive association between ultrafine particles and systemic inflammation but did not observe measurable respiratory symptoms (Clifford et al., 2018).

A similar result was reported between asthma and other respiratory diseases in children younger than 15 years and an increase in asthma-related hospital admissions when exposed to increasing concentrations of both fine and coarse PM (Tecer et al., 2008). Hence, evaluation of exposures of asthmatics to ultrafine particles in locations without combustion sources could be an important parameter in overall health evaluation.

Our information on asthma was based entirely on the residents' responses. Questions regarding asthma were not recorded specifically for each occupant but for an apartment as our analysis unit. We also did not collect information about the use of medications. In addition, the number of participants was relatively low. Thus, our findings should be verified in a larger study investigating IAQ and health. Despite these limitations, we showed an association between the airborne particle presence and recent occurrence of asthma.
2.5 Conclusions

Field studies are difficult but also essential to understand IAQ, given context-specific drivers including indoor and outdoor sources and conditions, building characteristics, and occupant behavior. This pilot-level study explored the potential to integrate infrared thermography with laser scanning, IAQ measurements, and resident interviews in order to provide a more comprehensive and faster assessment of building structural conditions and relate them to indoor environmental parameters and residents' well-being, such as asthma episodes in the past 12 months than would be possible with just one separate data stream. Our findings suggest that missing insulation is conducive for ultrafine particle penetration and accumulation from outdoors to indoors. At the same time, we recognize that the study evaluated a limited number of buildings and individual apartments. Despite this limitation, this pilot study demonstrates the feasibility of integrating different and sophisticated techniques into the investigation of building conditions, IAQ, and residents' health. Future studies should apply and investigate this methodology on a larger scale. Also, we found that only one of the building deficiencies determined by the infrared thermography with laser scanning, i.e., missing insulation, correlated with IAQ parameters and residents' health. Larger studies could show correlations with other types of building deficiencies as well. In summary, we believe that the data obtained in this pilot study will encourage other researchers to integrate various techniques in their investigations of buildings, IAQ, and residents' health so that a more comprehensive relationship between building performance, IAQ, and residents' health can be developed.

2.6 Acknowledgments

US Department of Housing and Urban Development (HUD), NJHHU0019-13. The funding sponsor had no role in the design of the study, the collection, analysis, or interpretation of data, in the writing of the manuscript, and in the decision to publish these results. The authors would like to expresses their appreciation to WHEDco for facilitating access to the buildings and to the participants who graciously allowed us to access their apartments.

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Figure 2-1. Field study sites. a) Building 1 and b) Building 2 (Photo credits: Google Maps and WHEDco: Women's Housing and Economic Development Corporation; Bronx, NY).



Figure 2-2. (a) Stitching of indoor infrared images; (b) Results of infrared temperaturebased segmentation



Figure 2-3. (a) 3D thermal point cloud model; (b) Segmented 3D point cloud

Parameter	Indoor/ Outdoor	Resolution	Instrument	Size	Operating	Flow	The accuracy	Reference	Indoors	
	locations			detection	Range	rate	of operating	from	studies with	
				limit			parameters	manufactur	similar use of	
							1		•	
								ers	instruments	
Temperature and Relative	Living room/ Courtyard	1 min	IAQ-Calc Indoor Air Quality	NA	32 to 140°F (0 to	NA	$\pm 1.0^\circ F~(\pm 0.5^\circ C)$ and	(TSI Inc)	(Ahmed et al., 2015;	
Humidity			Meter 7525		60°C) and 5% to 95%		±3.0% RH		Fsadni et al., 2018)	
					RH					
Particle number concentration	Living room/ Courtyard	1 min	P-Trak Ultrafine Particle	20 nm – 1 µm	0 to 5 x 10 ¹¹	0.1 L/min	Not mentioned by	(TSI Inc)	(Glytsos et al., 2010;	
			Counter 8525		particles/m3		manufacturer		J. Kearney et al.,	
									2011; Matson, 2005;	
									Rundell, 2003; Y.	
									Zhu et al., 2005)	
Particle number concentration	Living room/ Courtyard	1 min	AeroTrak Handheld Optical	0.3 to 25 µm	0 to 2.1 x 10 ⁸	2.83 L/min	\pm 5% of sampling	(TSI Inc)	(A. Chen et al., 2016;	
with size distribution			Particle Counter 9306		particles/m3		flow		Kim et al., 2017;	
									McGarry et al., 2011)	
Particle mass concentration	Living room/ Courtyard	1 min	Dustrak DRX Aerosol monitor	0.1 to 15 µm	0.001 to 150 mg/m ³	3.0 L/min	± 5% of sampling	(TSI Inc)	(Buonanno et al.,	
with size fractions			8534				flow		2011; Stabile et al.,	
									2017; Tong et al.,	
									2018; Zuocheng	
									Wang et al., 2016)	
PM _{2.5} mass concentration	Living room/ Courtyard	24 hours	Personal Modular Impactor with	PM _{2.5} size	NA	3.0 L/min	\pm 5% of sampling	(SKC Inc, n.d.)	(Amaral et al., 2015;	
			$2.5~\mu m$ cut size and $2~\mu m$ pore	fraction			flow		Mohammadyan &	
			size 37 mm PTFE filter						Ashmore, 2005;	
			Microbalance Mettler Toledo	NA	0.001 to 5100 mg	NA	$\pm \ 2 \ \mu g$ (up to 500 mg)	(Mettler-Toledo,	Zuocheng Wang et	
			(MT-5)				$\pm~4~\mu g$ (above 500	1999)	al., 2016)	
							mg)			

 Table 2-1. Parameters and specifications of instruments used in the study.



Figure 2-4. Flow chart of methodology from recruitment of residents to parameters measured for the three data streams.



Figure 2-5. Missing insulation on exterior walls indicated by the cooler colors (dark purple/black) in the infrared images.

Group		Ν	Mean	Median	Minimum	Maximum
			(Std.	(Std. Dev.)		
			Dev.)			
"Low"	Missing		3.24	2.22	0.45	9.31
(Missing	insulation	14	(2.97)	(2.97)		
insulation	(ft ²)					
below	Missing		1.25	0.77	0.13	3.65
5%)	insulation		(1.18)	(1.18)		
	(%)					
"High"	Missing		26.48	29.86	13.0	36.53
(Missing	insulation	6	(10.73)	(10.73)		
insulation	(ft ²)					
above	Missing		10.98	9.91	5.25	19.62
5%)	insulation		(5.54)	(5.54)		
	(%)					

Table 2-2. Descriptive statistics of missing insulation levels in both ft² and percentage of the wall area.

Table 2-3. Particulate matter concentrations and their indoor/outdoor ratios. Number concentrations were measured for 45-60 min using direct-reading instruments, while PM_{2.5} mass concentrations were measured for 24 hours using filter sampling; the latter is denoted by *.

Variable	Ν	Median (25 th , 75 th	Minimum	Maximum
		percentiles)		
Number concentration of ultrafine particles (<300	20	1.50 x 10 ¹⁰	2.64 x 10 ⁹	9.86 x 10 ¹⁰
nm) (#/m ³)		$(7.31 \times 10^9, 2.72 \times 10^{10})$		
Number concentration of particles >300 nm (#/m ³)	20	2.19 x 10 ⁷	1.38 x 10 ⁶	5.53 x 10 ⁸
		(9.22 x 10 ⁶ , 5.33 x 10 ⁷)		
Mass concentration of PM _{2.5} particles ($\mu g/m^3$) *	20	28.37 (19.07, 57.61)	7.23	96.29
Mass concentration of particles >PM _{2.5} (µg/m ³)	20	24.57 (16.01, 37.87)	7.51	45.26
Indoor/Outdoor ratio of ultrafine particle number concentration (<300 nm)	20	0.85 (0.57, 1.47)	0.35	13.14



Particle number concentration (#/m3) in apartments stratified by missing insulation





Figure 2-6. Airborne particle characteristics in investigated apartments stratified by missing insulation groups: "low" (n=14) and "high" (n=6). a. and b: Number concentration of particles smaller and larger than 300 nm, respectively. c. and d: Mass concentration of PM_{2.5} particles and larger particles, respectively. The asterisk (*) represents a statistically significant difference (p<0.05) between the groups. The upward facing triangles and downward facing triangles represent 1st and 99th percentile of the data, respectively; the whiskers represent 1.5x of interquartile range; the square represents the mean; the lower, middle and upper lines of each box plot are 25th percentile, median, and 75th percentile of the data, respectively.



Figure 2-7. The indoor/outdoor ratio of number concentration of ultrafine particles stratified by missing insulation (%) groups. The dotted red line represents the indoor/outdoor ratio equal to 1. The asterisk (*) represents a statistically significant difference (p<0.05) between the groups. The upward facing triangles and downward facing triangles represent 1^{st} and 99^{th} percentile of the data, respectively; the whiskers represent 1.5x of interquartile range; the square represents the mean; the lower, middle and upper lines of the box plot are 25th percentile, median, and 75th percentile of the data, respectively.









Figure 2-8. Number concentration $(\#/m^3)$ of ultrafine particles stratified by levels of missing insulation (%) with respect to the presence of combustion sources. a. Apartments with no smoking reported ("low" group: n = 9, "high" group: n = 5). b. Apartments with no candles or incense burnt ("low" group: n = 6, "high" group: n = 2). c. Apartments with no smoking, candles or incense burnt ("low" group: n = 5, "high" group: n = 2). d. Smoking reported indoors ("low" group: n = 5, "high" group: n = 1). e. Candles or incense burnt indoors ("low" group: n = 8, "high" group: n = 4). f. Smoking, candles or incense burnt indoors ("low" group: n = 9, "high" group: n = 4). The upward facing triangles and downward facing triangles represent 1st and 99th percentile of the data, respectively; the whiskers represent 1.5x of interquartile range; the square represents the mean; the lower, middle and upper lines of the box plot are 25th percentile, median, and 75th percentile of the data, respectively.

Resident Information							
Age (years)	Median (25 th , 75 th	Min. Max.					
	percentiles)						
Apartments with children younger than 18 years	11 (7, 13)	0.5 17					
(n = 13)							
Apartments with adults $(n = 20)$	36 (30, 52)	19 75					
Combustion sources							
Report (n= 20)	Yes, % (n)	No, % (n)					
Smoking indoors	30 (6)	70 (14)					
(subset of candles or incense burnt indoors)	84 (5 out of 6)	50 (7 out of 14)					
Candles or incense burnt indoors	60 (12)	40 (8)					
(subset of smoking indoors)	42 (5 out of 12)	13 (1 out of 8)					
Indoor combustion sources (smoking or	65 (13)	35 (7)					
candles/incense)							

 Table 2-4. Questionnaire data on the presence of combustion sources indoors and residents' age.



Figure 2-9. Association between missing insulation (%) and apartment location. a. Association between corner apartments (7/20 apts.) and the level of missing insulation (%). b. Association between apartments located on floors above 5 (11/20 apts.) and the level of missing insulation.

Report (n= 20)	Yes,	%	No,	%
	(n)		(n)	
History of asthma for anyone in the apartment)	40 (8))
Reported asthma attacks for anyone in the apartment in the last	30 (6)		70 (14	4)
12 months				

 Table 2-5. Questionnaire data on asthma prevalence.



Reported asthma attacks in the last 12 months

Figure 2-10. a. Missing insulation (%) in apartments stratified by reported asthma attacks in the last 12 months. b. Number concentration of ultrafine particles stratified by reported asthma attacks in the last 12 months. c. PM_{2.5} mass concentration (μ g/m³) stratified by reported asthma attacks in the last 12 months. d. Association between smoking indoors and reported asthma attacks in the last 12 months. The asterisk (*) represents a statistically significant difference (p<0.05) between the groups. The upward facing triangles and downward facing triangles represent 1st and 99th percentile of the data, respectively; the whiskers represent 1.5x of interquartile range; the square represents the mean; the lower, middle and upper lines of the box plot are 25th percentile, median, and 75th percentile of the data, respectively.

CHAPTER 3. PRESENCE AND VARIABILITY OF CULTURABLE BIOAEROSOLS IN THREE MULTI-APARTMENT RESIDENTIAL BUILDINGS WITH DIFFERENT VENTILATION SYSTEMS IN THE NORTHEASTERN US

3.1 Abstract

Bioaerosol concentrations in residential buildings located in the Northeastern US have not been widely studied. Here, in 2011-2015, we studied the presence and seasonal variability of culturable fungi and bacteria in three multi-apartment residential buildings and correlated the values with building' ventilation system types and environmental parameters. A total of 409 indoor and 86 outdoor samples were taken. Eighty-five percent of investigated apartments had culturable fungi indoor-outdoor (I/O) ratios below 1, whereas in 56% of the apartments, these ratios for culturable bacteria were above 1, suggesting minimal indoor sources of fungi and prominence of indoor sources of bacteria. Culturable fungi I/O ratios in apartments serviced by central heating, ventilation and airconditioning (HVAC) system were lower than those in apartments with window AC. Interestingly, the type of ventilation system did not have a significant effect on indoor culturable bacteria. Also, residents in apartments with central HVAC did not experience extreme dew point (DP) values. A significant positive association was determined between indoor DP levels and indoor culturable fungi (p < 0.001) and bacteria (p < 0.001), regardless of ventilation type. We conclude that building infrastructure, seasonality, and indoor sources are major factors affecting indoor bioaerosol levels in residential buildings.

Keywords

Bioaerosols - Multi-Apartment Residential Buildings - Culturable Bacteria - Culturable
 Fungi - HVAC systems - Dew point
3.2 Introduction

Bioaerosols are airborne particulate matter of biological origin and vary in size from 0.3 µm to 100 µm. A wide range of bioaerosols exists, such as bacteria, fungal spores, fragments of fungal mycelium, viruses, pollens, and their by-products (toxins) (Cox & Wathes, 1995; Crook & Sherwood-Higham, 1997; Mainelis, 2019; Salem & Gardner, 1994). They are emitted into the air by natural and anthropogenic sources, including humans, animals, plants, heating, ventilation, and air-conditioning systems (HVAC), resuspension of dust and soil, waste treatment facilities, agricultural activities, and other sources (Abdel Hameed et al., 2009; Kummer & Thiel, 2008; Prussin et al., 2015; Yassin & Almouqatea, 2010).

Exposure to bacterial and fungal aerosols has been associated with numerous health effects, including respiratory diseases, such as asthma, allergic rhinitis, hypersensitivity pneumonitis (Douwes et al., 2003; Hellebust et al., 2018; Humbal et al., 2018; Srikanth et al., 2008). *Cladosporium, Penicillium, Stachybotrys spp.*, and *Aspergillus* fungal genera are common species causing allergic responses and infections (Garaga et al., 2019; Karimpour Roshan et al., 2019). Associations of wheezing, chronic cough, and subjective respiratory symptoms with damp/moldy surroundings have also been well-established (Fung & Hughson, 2003). Children's respiratory and immune systems are vulnerable to bioaerosol exposure even before birth (Sly & Bush, 2019).

Since we spend approximately 90% of our time indoors, including approximately 70% of the time in our homes (de Kluizenaar et al., 2017), it is vital to investigate factors that affect the presence of indoor bioaerosols in order to understand and prevent potential negative health impacts due to bioaerosol exposures. Moreover, bioaerosol concentrations

indoors can be ten-fold higher compared to outdoor concentrations (Prussin & Marr, 2015). Indoor bioaerosol exposures have been studied in occupational settings, including composting facilities (Bünger et al., 2000; Domingo & Nadal, 2009), agriculture, livestock, and food production facilities (Fischer & Dott, 2003; S.-A. Lee et al., 2005), and the waste recycling industry (Lavoie et al., 2006; Marchand et al., 1995; Poulsen et al., 1995). Other indoor spaces, such as single-family homes (DeKoster & Thorne, 1995; Fabian et al., 2005; T. Lee et al., 2006), offices (Hsu et al., 2012; H. Zhu et al., 2003), hospitals (Lai et al., 2014; Nourmoradi et al., 2012), schools (Godwin & Batterman, 2007; Hussin et al., 2011; Nevalainen et al., 1991) have also been investigated worldwide. While there is a substantial body of literature on bioaerosols in individual homes (DeKoster & Thorne, 1995; Fabian et al., 2005; T. Lee et al., 2006), limited studies have focused on multi-apartment residential buildings. Among those, Moon et al. investigated 25 households for culturable bacteria and fungi in high-rise apartment buildings in Korea (Moon et al., 2014). Mentese et al. studied apartments and public spaces in Turkey for culturable bioaerosols in summer and winter (Mentese et al., 2012). A study by Lee and Jo in 2006 investigated the influence of seasons, room location, and apartment floors on indoor and outdoor bioaerosol concentrations at a high-rise apartment building in Korea (J.-H. Lee & Jo, 2006). However, to best of our knowledge, few detailed studies of bioaerosol exposure in multi-apartment residential buildings in the United States (US) are available, even though in 2017 up to 35% of the US population lived in apartment buildings (Statista, 2019). Thus, there is a knowledge gap regarding residents' exposure to bioaerosols in multi-apartment residential buildings. Such exposures could be different from more frequently studied exposures in single homes due to the higher population density in multi-apartment buildings and the overlap and variety

of sources, including the distribution of dust and bioaerosols from common centralized heating and cooling systems (Horner, 2006; Mirhoseini et al., 2016).

There are multiple factors that affect bioaerosol presence indoors, including bioaerosol penetration from outdoors and their sources indoors (Burge, 1995; Moon et al., 2014; Prussin & Marr, 2015). Among the latter, anthropogenic sources including household dust, dead human skin (Al-Hunaiti et al., 2017; Cox & Wathes, 1995), indoor human activities (Heo et al., 2017), and the presence of pests and pets (Cox & Wathes, 1995; Gereda et al., 2001) can affect the composition and concentration of indoor bacteria. Indoor fungal aerosols typically have an outdoor origin, but could also have a substantial contribution from moldy building materials or be carried into indoor space by humans on clothing and skin (Cox & Wathes, 1995; William W Nazaroff, 2016; Prussin & Marr, 2015). Water provides enrichment potential for microorganism growth, and buildings with water damage are directly correlated with high indoor fungal aerosols (Fabian et al., 2005; Nevalainen et al., 1991). An increase in relative humidity indoors has been associated with higher indoor culturable fungi levels, while indoor bacteria have often been correlated with higher temperatures and warmer seasons (Balasubramanian et al., 2012; DeKoster & Thorne, 1995; T. Lee et al., 2006; Mentese et al., 2012).

Building design elements, such as HVAC type and their maintenance, could also affect bioaerosol levels. A study conducted in 37 hospitals in Taiwan reported higher culturable fungi concentrations in buildings with window-type air conditioning compared to buildings with central air conditioning (Jung et al., 2015). Excess moisture indoors can lead to microbial growth, but it can be controlled with efficient ventilation and indoor temperature maintenance along with proper cleaning practices (Heseltine et al., 2009). A study conducted in an apartment in Singapore related high indoor culturable concentrations of bacteria and fungi to the use of air conditioners without proper maintenance and cleaning of the filter. (Balasubramanian et al., 2012).

The collective effect of the multiple factors mentioned above can have a significant impact on occupants' exposure to indoor bioaerosols. Hence, a holistic approach must be taken to study bioaerosols indoors with the inclusion of multiple factors such as spatiotemporal variability, fungi, and bacterial diversity, and building design elements. The primary goal of this study was to fill the knowledge gap regarding the exposures to culturable bioaerosols in multi-apartment buildings and investigate the quantitative and seasonal variability of culturable bacteria and fungi in three multiapartment buildings in the Northeastern US, taking into account the effect of seasons and building characteristics. The building characteristics included their ventilation systems, location, and dew point (DP) profiles. These three buildings were part of a broader indoor air quality (IAQ) studies conducted between 2011 to 2015 that investigated factors that affect residents' exposures to indoor pollutants in multiphase interventional and longitudinal studies, including the investigation of the residents' perception of their health and building conditions (Hewitt et al., 2016; Jordán-Cuebas et al., 2018; Patton et al., 2016; Thomas et al., 2019; Zhaojun Wang et al., 2018; Xiong et al., 2015). As part of those studies, we found that ultrafine and PM_{2.5} particle concentrations were associated with building deficiencies, presence of indoor combustion sources including smoking and burning of candles/incense, and occupant behavior (Patton et al., 2016; Thomas et al., 2019; Xiong et al., 2015). In addition to IAQ parameters, end-use water consumption was measured and modeled to predict potential leaks in apartments (Jordán-Cuebas et al.,

2018). As part of the IAQ investigations, culturable bioaerosol data were also collected. Hence, this part of the study and the manuscript uses this opportunistic data set and focuses on culturable bioaerosols and environmental and building variables that affect their concentrations.

3.3 Materials and Methods

3.3.1 Study Buildings and Measurement Timeline

Bioaerosol measurements were performed in Building 1 (B1), Building 2 (B2), and Building 3 (B3) between 2011 to 2015 (Table 3-1), according to the timeline shown in Figure 3-1. Our study invited all residents from the three buildings to participate via brochures and communications from the building management, and the recruitment is described in more detail in earlier publications (Hewitt et al., 2016; Jordán-Cuebas et al., 2018; Patton et al., 2016; Thomas et al., 2019; Wang et al., 2018; Xiong et al., 2015). B1 is an Energy Star certified residential building with an urban rooftop garden and a total of 128 apartments on six and seven floors. B1 has a hot water baseboard heating supplied by a centralized boiler and natural air ventilation, predominantly using open windows. A previous study on B1 reported that approximately 73% of the apartments had at least one window air conditioning (AC) unit. The majority of the households had an annual income of less than \$20,000. (Patton et al., 2016) The total of 15 different apartments was sampled in B1 in three separate campaigns (referred to as C1, C2, and C3 in Figure 3-1), and 7 apartments were the same for all three campaigns. Since Building 1 was studied during three different campaigns at very different time points (e.g., 2011, 2013, and 2015), we also investigated these campaigns separately, i.e., B1-C1, B1-C2, and B1-C3 (Appendix B-1 and B-2). B2 is a LEED (Leadership in Energy and Environmental Design) EB

(Existing Building) Platinum certified luxury residential building with 27 floors and located in a waterfront neighborhood, with households having an annual income of more than \$200,000 (Patton et al., 2016). B2 has a central HVAC that supplies 100% conditioned outdoor air, including filtration by a Minimum Efficiency Reporting Value (MERV) 7 filter followed by a MERV 14 filter on air intakes and dehumidification to 30% relative humidity (RH) at 65 °F (18.3 °C) in the summer and humidification to 50% RH at 76 °F (24.4 °C) in the winter (Xiong et al., 2015). Fan-coil units fitted with MERV 11 filters provided heating and cooling independently from the supply air. These MERV filters were expected to remove $\geq 60\%$ of all particles and 90% of particles $< 0.02 \ \mu m \text{ or } \geq 1 \ \mu m$ (Patton et al., 2016). The building operator and the investigators rarely recorded windows to be open; hence we assumed that the central HVAC was switched on continuously. A total of 18 apartments were sampled in this building. B3 is a former hospital retrofitted into a residential building with a total of 132 apartments on ten floors. It also includes a commercial kitchen and a child development center. The apartments had a radiant baseboard heating system from a centralized boiler and small wall AC units (MacDonald, 2015). The annual household income in B3 was similar to that of B1. Fifteen apartments were sampled in B3. The terms "apartment" and "household" are used interchangeably in this manuscript.

3.3.2 Seasons

In each building, samples were collected during several seasons, as shown by color-codes in Figure 3-1: Spring (Sp) in green, Summer (Su) in yellow, Fall (F) in orange, and Winter (W) in blue. The sampling days were assigned seasons based on the winter/summer solstice days and spring/fall equinox days for the particular year, using data from the United States Naval Observatory ¹⁷. B1-C1 was sampled from July 2011 to May 2012 during Su, F, and Sp seasons. B1-C2 was sampled from August 2012 for a year, e.g., during all four seasons. B1-C3 was sampled in March of 2015 and included both W and Sp seasons. B2 was sampled from February to December of 2011 and included all four seasons. Samples were collected in B3 from June to August of 2014 during Sp and Su seasons.

3.3.3 Measurements

Bioaerosol concentration and environmental parameters, e.g., temperature and relative humidity, were measured using a collector and real-time monitors, respectively. Equivalent outdoor measurements were taken on all the days when indoor measurements were performed. All devices were within one year of their calibration by the manufacturer and were considered to be in calibration. Each sampling day consisted of measuring bioaerosols in the living room of one to four apartments and an equivalent outdoor sample. Table 3-2 describes the number of samples collected during the study for each variable stratified by the three buildings and by seasons for each building. In summary, the study through the 5-year period collected 409 indoor and 86 outdoor samples. Sampling and sample analysis details are described below.

3.3.3.1 Culturable Bacteria and Fungi Concentrations

Concentrations of culturable bacteria and fungi were measured using a portable SAS Super 180 air sampler (Bioscience International, Rockville, MD) operated at 180 L/min for 3 minutes. While the terms "fungi" and "mold" have been used interchangeably in the bioaerosol literature (Andersson et al., 1997; Nevalainen et al., 1991; Pasanen et al., 1991; US EPA, 2014b), we are using the term "fungi" to encompass both fungal spores and mold. Trypticase soy agar (Difco, Becton, Dickinson and Co., Sparks, MD) with fungicide

cycloheximide (50 µg/mL; Fisher Scientific Company Ltd., Hanover Park, IL) and malt extract agar (Difco, Becton, Dickinson, and Co.) were used as sampling media for bacteria and fungi, respectively. The sampler was wiped down with alcohol prep pads (cat. no.: 22-363-750; Fisherbrand, Fisher Scientific Company Inc.) prior to and in between samples. After sampling, the plates were incubated at room temperature 68–72 °F (20 – 22 °C) in an incubator with a water beaker to prevent desiccation for three and five days for bacteria and fungi, respectively. The resulting colony forming units (CFU) were counted, and their counts converted to airborne concentrations after applying a positive hole correction factor. The factor accounts for the probability that more than one colony-forming bioaerosol could pass through the same impactor nozzle, of which there were 401, and form a joint colony (Macher, 1989). Three samples were taken at each apartment and average concentrations were calculated for each sampling medium. Both field and media blanks were included for each sampling day and any CFU counts on the blanks were subtracted from sampling data.

3.3.3.2 Temperature, Relative Humidity, and Dew point

A direct reading instrument IAQ-Calc Indoor Air Quality Meter (model 7525, TSI Inc.) was used to measure and log the temperature (°F) and relative humidity (RH, %) for 45 – 60 min in each apartment. The data logging interval was 1 min and an average value was calculated for each apartment. DP was calculated from the temperature and relative humidity readings using the formula given by Lawrence (M. G. Lawrence, 2005), with constant values of the equation derived from Alduchov and Eskridge (Alduchov & Eskridge, 1996).

3.3.4 Statistical Analysis

SPSS v26.0 (IBM, Armonk, NY) and OriginPro 2018 (OriginLab, Northampton, MA) were used to analyze and illustrate the data, respectively. The data were tested for normality using the Shapiro-Wilk test. Since the p-values were below 0.05 for the entire dataset, which indicated a failed normality test for the independent variables, non-parametric tests were used. We chose not to log-transform data as that often can provide statistical results that are irrelevant for the original data and increase the difficulty in data analysis results; hence we proceeded with non-parametric tests (Feng et al., 2014). The statistically significant differences among three or more groups were determined by the Kruskal-Wallis H Test (χ^2), followed by the Games-Howell post-hoc analysis to identify the pairs that differed statistically significantly. Groups with p values of less than 0.05 were considered to be significantly different and groups with p-values of less than 0.1 were considered borderline significantly different. The mean difference between any two groups was determined by the Mann-Whitney U test. Spearman rank correlation coefficient, rs, was used to determine associations between variables, i.e., associations between culturable bioaerosols with DP, temperature, and RH. Values of $r_s < 0.3$, 0.3 - 0.6, and > 0.6 were considered to have weak, moderate, and strong associations, respectively (Akoglu, 2018). In order to simplify the data analysis, each apartment was considered as an independent data point even though several apartments in B1 (seven, to be exact) were sampled in all three campaigns and during all seasons. When analyzing the results, the data from the three campaigns in B1 were pooled together. To investigate the effect of ventilation systems types, the data was stratified into two apartment groups: "Central HVAC" and "Window AC." "Central HVAC" was comprised of apartments with central cooling and heating (i.e.,

B2). "Window AC" was comprised of apartments with window AC units with natural ventilation and radiator heating (i.e., B1 and B3). The extent of data dispersion within the repeatedly-sampled apartments of B1 was estimated by using the coefficient of variation (CV). Since the data were not normally distributed, they are presented in the text and tables as median values, and 25th and 75th percentiles.

3.4 Results and Discussion

3.4.1 Indoor Culturable Fungi

Concentrations in buildings without seasonal stratification

The concentrations varied significantly between the three buildings ($\chi^2(2) = 77.261$, p < 0.001), according to the Kruskal-Wallis *H* test. The lowest concentrations were measured in B2 (median: 27 CFU/m³) and they were significantly lower than concentrations in B1 (p < 0.001; median: 71 CFU/m³) and B3 (p = 0.001; median: 156 CFU/m³). Between the two buildings with window ACs and natural ventilation ("window AC" apartments), fungi concentrations were significantly lower in B1 compared to B3 (p = 0.034).

Seasonal effect within each building

The indoor culturable fungi concentrations stratified by four seasons for the three buildings are presented in Figure 3-2a and Table 3-3.

B1: The concentrations varied significantly by the seasons ($\chi^2(3) = 55.960$, p < 0.001). Concentrations in winter (median: 21 CFU/m³) were significantly lower than during the other seasons (p < 0.05). The highest median concentration of 111 CFU/m³ was measured in summer, and concentrations in summer were significantly higher than the concentrations in fall (median: 64 CFU/m³; p = 0.021). Even though the median concentration in summer

was ~2x of that in spring (median: 58 CFU/m³), they were not significantly different (p = 0.620). Furthermore, variations between the three campaigns in B1 are described in Appendix B-1 and shown in Figure B-1a. Overall, the concentrations differed by the seasons during B1-C1 and B1-C2, whereas concentrations in spring and winter during B1-C3 measurements were similar to each other. The latter could be explained by collection of samples on days bordering the spring equinox.

B2: The concentrations measured in B2 during winter, with a median of 5 CFU/m³, were the lowest compared to the other seasons. It was significantly ($\chi^2(3) = 48.595$, p < 0.001) lower than fall concentrations (median: 43 CFU/m³; p = 0.007), and borderline significantly lower than concentrations in spring (median: 19 CFU/m³; p = 0.068) and summer (median: 36 CFU/m³; p = 0.064). Concentrations during spring, summer, and fall were not different (p > 0.1), probably due to the use of the buildings' centralized HVAC.

B3: Concentrations in B3 during spring and summer seasons, with median values of 149 CFU/m^3 and 162 CFU/m^3 respectively, were not statistically different from each other (U= 35.0, p = 0.433). The samples were collected on days fairly close to the summer solstice, and, therefore, a difference in concentrations between the two seasons was not detected. *Differences between the three buildings during the same seasons*

Spring: The concentrations between the three buildings differed significantly ($\chi^2(2) = 23.797$, p < 0.001). B2, with the lowest median concentration of 19 CFU/m³, had significantly lower concentrations than in B1 (median: 58 CFU/m³: p = 0.001). The highest median concentration was measured in B3 (median: 149 CFU/m³). However, the concentrations in B3 were only borderline significantly higher than concentrations in B2

(p = 0.086). The two buildings without central AC, i.e., B1 and B3, had comparable concentrations (p = 0.776).

Summer: The concentrations differed between the three buildings ($\chi^2(2) = 50.018$, p < 0.001), a result similar to that in spring. B2 had the lowest median concentration (median: 36 CFU/m³) and its indoor fungi concentrations were significantly lower than those in B1 (median: 111 CFU/m³: p = 0.032) and B3 (median: 162 CFU/m³: p = 0.036). B1 and B3 had comparable concentrations (p = 0.810).

Fall: The two buildings with (B2) and without (B1) central AC had significantly different concentrations, according to the Mann-Whitney U test (U = 584.0, p = 0.009). Concentrations in B2 (median: 43 CFU/m³) were almost half of B1 concentrations (median: 64 CFU/m³).

Winter: The concentrations in the two buildings, B1 and B2, were significantly different from each other (U = 350.0, p < 0.001), and this result was similar to the fall season. Concentrations in B1 (median: 21 CFU/m³) were 4x higher than concentrations in B2 (median: 5 CFU/m³).

Context of fungi levels

The fungal concentrations from our study are similar to what was reported by other studies in the US and other countries. In the US, Lee et al. (2006) reported a geometric mean of 88 CFU/m³ in six single family homes. Shelton et al. studied IAQ in 1717 buildings across the US and measured a median concentration of 80 CFU/m³ (Shelton et al., 2002). The Building Assessment Survey and Evaluation (BASE) study measured 100 US office buildings and reported a mean value of 100 CFU/m³ (Tsai et al., 2007). A study conducted in China reported culturable fungi concentrations in 454 residences with an average value of 300 CFU/m³ (X. Wang et al., 2016), which was similar to the higher values in our study. Mirhoseini et al. reported a similar range of concentrations in offices, residences, dormitories, laboratories, and classrooms located in Iran (50 - 1060 CFU/m³) (Mirhoseini et al., 2016). In general, in our study, the concentrations of indoor culturable fungi were the lowest for the "central HVAC" apartments compared to the "window AC' apartments. Similarly, Wang et al. reported lower concentrations of indoor airborne fungi in indoor locations with mechanical ventilation compared to natural ventilation (X. Wang et al., 2016).

3.4.2 Culturable Fungi Indoor – Outdoor (I/O) Ratio

Ratios in the buildings without seasonal stratification

The overall I/O ratios ranged from 0.01 to 17.41, and 85% of all apartments (348/409 apartments) had I/O ratios below 1, indicating higher fungi concentrations outdoors compared to indoors and limited contribution from indoor sources. This result suggests the accumulation of outdoor fungi indoors and/or minimal contribution from indoor sources (Nevalainen et al., 1991; Ponce-Caballero et al., 2010). In general, this was the case for all three buildings and all four seasons. The culturable fungi I/O ratios differed between the buildings ($\chi^2(2) = 82.904$, p <0.001), in a way similar to the indoor culturable fungi concentrations. The lowest and highest median values were measured in B2 (median: 0.18) and B3 (median: 0.61), respectively. The I/O ratios in B2 were significantly lower than in B1 (p = 0.001; median: 0.53) and B3 (p = 0.030), indicating that a central ventilation system is an important building attribute to maintain lower indoor fungi concentrations compared

to outdoors. The I/O ratios in the "window AC" apartments, i.e., B1 and B3, were not different (p = 0.542).

Seasonal effect within each building

The culturable fungi I/O ratios stratified by the four seasons for the three buildings are presented in Figure 3-2b and Table 3-3.

B1: I/O ratios did not differ significantly during the four seasons ($\chi^2(3) = 10.806$, p = 0.881). The highest and lowest median I/O ratios of 0.62 and 0.35 were measured in summer and winter, respectively. I/O ratios in spring (median: 0.52) and fall (median: 0.50) were nearly the same. Variations between the three campaigns in B1 are shown in Figure B-1b and described in Appendix B-1. Overall, 81% of the apartments in B1 (187/230 apartments) had median I/O ratios below 1 for the three campaigns and seasons, which was a similar percentage compared to the other buildings as well. However, the I/O ratios within each campaign differed by seasons.

B2: The I/O ratios were generally below 1, excluding the outliers shown in Figure 3-2b. All four seasons had non-different I/O ratios in ($\chi^2(3) = 4.006$, p = 0.261). This result indicates higher outdoor concentrations, especially in fall with a median I/O of 0.13. The median I/O ratios in spring, summer, and winter were 0.20, 0.16, and 0.27, respectively.

B3: The median I/O ratios of B3 were similar to that in the other two buildings where the median ratios were also below 1. The median I/O in spring and summer seasons were 0.56 and 0.63, respectively, with no significant difference in I/O ratios between the seasons (U= 34.0, p = 0.400).

Spring: The I/O ratios for the three buildings differed significantly between each other $(\chi^2(2) = 20.566, p < 0.001)$. The building B2 (median: 0.20) had the lowest I/O ratios, which was analogous to the indoor culturable fungi comparisons. I/O ratios in B1 (median: 0.52; p = 0.090) and B3 (median: 0.56; p = 0.077) were higher than those in B2, though only with borderline significance. B1 and B3 had similar I/O ratios (p = 0.744).

Summer: The I/O ratios different in a way similar to the indoor culturable fungi concentrations in summer. The I/O ratios in the three buildings differed significantly from each other ($\chi^2(2) = 56.451$, p < 0.001). I/O ratios in B2 (median: 0.16) were significantly lower than that in B1 (median: 0.62; p = 0.002) and B3 (median: 0.63; p = 0.052), while the latter two buildings had non different values (p = 0.988).

Fall: The median I/O ratio in B2 (median: 0.13) was approximately 4x lower than the median I/O ratio in B1 (median: 0.50), and the I/O ratios for the two buildings differed significantly (U = 355.5, p < 0.001).

Winter: The two buildings, B1 and B2, had similar I/O ratios (U = 727.0, p = 0.370). This result for winter, where the I/O ratios in B2 (median: 0.27) were not significantly lower than B1 (median: 0.35), was in contrast to the other seasons. We speculate that B2 had similar I/O ratios to B1 due to the moisture added in winter by the central HVAC system in B2.

Context of culturable fungi I/O ratios

The I/O ratios for the "central HVAC" apartments were the lowest for spring, summer, and fall seasons compared to the "window AC" apartments. Interestingly, the I/O ratios in

winter for the "central HVAC" apartments were similar to the "window AC" apartments. Humidification of the "central HVAC" apartments could have increased their I/O ratios in winter in contrast to the other seasons. Nonetheless, the "window AC" apartments also had their lowest I/O ratios in winter. We speculate that lower I/O ratios in winter are partially explained by lower outdoor temperatures and relative humidity, and buildings in the "window AC" apartments having less accumulation of indoor moisture compared to the other seasons due to its ventilation. Other studies reported similar culturable fungi I/O ratios. In the US, one study of six single homes showed a mean geometric I/O ratio of 0.66 (T. Lee et al., 2006). The IAQ investigation of 1717 buildings across the US yielded an I/O ratio equal to 1 or lower in 85% of the buildings, a percentage similar to our study. Moreover, another study in the Northeastern US reported I/O ratios similar to our study: from 0.1 to 0.5 (Shelton et al., 2002). The BASE study had a mean I/O ratio of 0.14 for 100 office buildings (all with HVAC systems) in the US (Tsai et al., 2007); this result was comparable to the median I/O ratio of 0.18 measured in the "central HVAC" apartments. A study of residential multi-apartment buildings in S. Korea reported culturable fungi I/O varying from 0.86 to 1.31 (Moon et al., 2014).

3.4.3 Indoor Culturable Bacteria

Concentrations in buildings without seasonal stratification

The concentrations varied between the three buildings ($\chi^2(2) = 3.475$, p = 0.088), but only with borderline significance. The lowest median concentration was measured in B3 (median: 214 CFU/m³), followed by in B2 (median: 219 CFU/m³). The median concentration of 235 CFU/m³ in B1 was significantly higher than that in B2 (p = 0.022).

Also, there was no difference in overall bacterial concentrations between the two buildings in the "window AC" apartments, i.e., B1 and B3 (p = 0.788).

Seasonal effect within each building

The indoor culturable bacteria concentrations for the three buildings stratified by seasons are shown in Figure 3-3a and Table 3-4.

B1: Concentrations in B1 did not change significantly for the four seasons ($\chi^2(3) = 4.801$, p = 0.187). The two lowest median concentrations were measured in fall (median: 200 CFU/m³) and winter (median: 235 CFU/m³). Spring and summer had higher concentrations with medians of 241 CFU/m³ and 278 CFU/m³, respectively. Additionally, the three campaigns in B1 are illustrated in Figure B-2a and described in Appendix B-1. Overall, the concentrations were similar between the three campaigns and the seasons.

B2: The concentrations over the four seasons were significantly different from each other $(\chi^2(3) = 30.599, p < 0.001)$, with the lowest median concentration measured during winter (median: 135 CFU/m³). In winter, the concentrations were significantly lower than those in spring (median: 183 CFU/m³: p = 0.001), and fall (median: 225 CFU/m³: p = 0.010), while the concentrations in summer (median: 276 CFU/m³: p < 0.001) were ~2x higher than those in winter. Concentrations in summer were also significantly higher than those in spring (p = 0.005), while fall concentrations were not different to those in spring (p = 0.403) or summer (p = 0.616).

B3: Concentrations in B3 did not differ between the two seasons (U = 35.0, p = 0.866): median concentration of 169 CFU/m³ in spring and median concentration of 229 CFU/m³

in summer. The spring and summer samples were collected on days bordering the summer solstice and hence no difference was determined.

Differences between the three buildings during the same seasons

Spring: Concentrations in spring did not differ significantly between the three buildings $(\chi^2(2) = 2.173, p = 0.337)$. The median values ranged from 169 CFU/m³ to 241 CFU/m³ for the three buildings, with the lowest concentration measured in B3 and the highest concentration in B1. Though B2 had a central HVAC, its median concentration (183 CFU/m³) was higher than in B3.

Summer: The concentrations did not differ between the three buildings ($\chi^2(2) = 2.165$, p = 0.339); this result was analogous to spring. The lowest median concentration of 229 CFU/m³ was measured in B3, whereas similar median concentrations of 278 CFU/m³ and 276 CFU/m³ were measured in B1 and B2, respectively.

Fall: Concentrations in B1 (median: 200 CFU/m³) and B2 (median: 225 CFU/m³) were not different from each other (U = 762.0, p = 0.289) regardless of the building's ventilation system. **Winter:** The median concentration measured in B1 (median: 235 CFU/m³) was almost 2x higher than in B2 (median: 135 CFU/m³) and the difference was statistically significant (U = 420.0, p < 0.001); this result was different from the other three seasons for indoor culturable bacteria.

Context for culturable bacteria levels

The indoor culturable bacteria concentrations measured in this study were similar to the "low" levels (10 to 1000 CFU/m³) reported in the review paper by Blais-Lecours et al. in schools, homes, and offices (Blais-Lecours et al., 2015). The BASE study conducted in

100 public and commercial office buildings in the US had a mean concentration of 280 CFU/m³, which was slightly higher than the mean concentration of 228 CFU/m³ in our study. Moon et al. measured concentrations in residential apartments similar to our study (geometric mean: 673 CFU/m³) and observed that culturable bacteria concentrations peaked during summer (Moon et al., 2014). Similar seasonal variations (10 to 3500 CFU/m³) were also reported by Moschandreas et al. in residences located in the Midwestern US (Moschandreas et al., 2003). The three buildings, regardless of their ventilation system, had similar concentrations of culturable bacteria during spring, summer, and fall. A similar result was published in Finnish homes where the mechanically and naturally ventilated buildings did not affect indoor bacteria concentrations (Reponen et al., 1989).

3.4.4 Culturable Bacteria Indoor-Outdoor (I/O) Ratio

Ratios in the buildings without seasonal stratification

Contrary to the culturable fungi I/O ratio, the culturable bacteria I/O ratio was typically above 1 for all buildings regardless of the ventilation system. Specifically, 56% of all apartments (227/409 apartments) had culturable bacteria I/O ratios above 1, with median values for the three buildings ranging from 1.13 to 1.38. The I/O ratios did not significantly differ between the three buildings ($\chi^2(2) = 1.258$, p = 0.533). However, B2 with central AC had the highest median I/O ratio of 1.36. The two buildings in the "window AC" apartments had similar median I/O ratios (B1: 1.13; B3: 1.16). The observed I/O ratios > 1 usually indicate the contribution of indoor and outdoor sources to the presence of bacteria (Faridi et al., 2015; Reponen et al., 1989). We speculate that indoor sources in these buildings including residents and their activities, pets, frequency of cooking and cleaning, resuspension of biological particles, and contribution of outdoor bacteria through natural ventilation, played a dominant role in the indoor accumulation of bacteria (Heo et al., 2017; Miletto & Lindow, 2015; William W Nazaroff, 2016; Prussin & Marr, 2015).

Seasonal effect within each building

The culturable bacteria I/O ratios for the three buildings stratified by seasons are shown in Figure 3-3b and Table 3-4.

B1: The I/O ratios in B1 did not differ significantly during the four seasons ($\chi^2(3) = 18.401$, p = 0.639), with median values ranging from 0.82 to 1.42. The I/O median ratio was highest during summer (median: 1.42) followed by fall (median: 1.15). There were almost equal concentrations of bacteria indoors and outdoors during Spring (median I/O: 1.03), while winter was the only season with higher outdoor concentrations compared to indoors (median I/O: 0.82). Differences between the three campaigns in B1 are shown in Figure B-2b and described in Appendix B-1. Overall, 55% of sampled apartments (127/230) had I/O ratios above 1 for the three campaigns and seasons, which was a similar percentage for all three investigated buildings. The I/O ratios between B1-C1 and B1-C2 differed by the seasons, but only with borderline significance (p < 0.1).

B2: Seasons in B2 had I/O ratios significantly different from each other ($\chi^2(3) = 18.149$, p < 0.001). Spring was the only season with a median value below 1 (median I/O: 0.76). Furthermore, Spring I/O ratios was significantly lower than that in summer (median: 1.47; p = 0.003), fall (median: 2.39; p = 0.006), and winter (median: 1.15; p = 0.049). The latter three seasons did not have significantly different I/O ratios (p > 0.05).

B3: Though the median I/O ratio during spring (median: 1.28) was higher than I/O in summer (median: 1.08), the difference was not significantly different (U = 34.0, p = 0.800).

Differences between the three buildings during the same seasons

Spring: The I/O ratios of the three buildings did not differ significantly during spring ($\chi^2(2) = 2,829$, p = 0.243). The buildings with the median I/O ratio close to 1 were B2 (median: 0.76) and B1 (median: 1.03). The highest median I/O ratio among the three buildings was measured in B3 (median: 1.28).

Summer: All median I/O ratios were above 1, and there were significant differences among buildings ($\chi^2(2) = 2.310$, p = 0.315). The lowest median I/O ratio was measured in B3 (median: 1.08). The other two buildings, with (i.e., B2) and without (B1) central AC, had similar I/O ratios with median values of 1.47 and 1.42, respectively.

Fall: The median I/O ratio in B2 (median: 2.39) was 2x higher than the median I/O ratio in B1 (median: 1.15), and the difference was significantly different (U = 501.5, p = 0.001).

Winter: B2 had a higher median I/O ratio than B1, i.e., 1.15 vs. 0.82, but the difference was borderline significant (median: 0.82) (U = 674.5, p = 0.083).

Context of culturable bacteria I/O ratio results

Similar to our study, Moon et al. measured culturable bacteria I/O ratios above 1 for all four seasons in 25 apartments located in S. Korea (Moon et al., 2014). A study in Turkey reported culturable bacteria I/O ratios ranging from 1.62 to 141.73 for a total of 120 indoor environments (Mentese et al., 2009). Public places and homes in Portugal were studied and showed higher indoor bacterial concentrations compared to outdoors, with I/O ratios

ranging from 0.62 and 40.8 (Madureira et al., 2015; Pegas et al., 2010). Interestingly, in our study, the I/O ratios of culturable bacteria during spring and summer seasons had similar values between the "window AC" and "central HVAC" apartments, and the "central HVAC" apartments had higher culturable bacteria I/O during fall and winter compared to the "window AC" apartments. Even though the "central HVAC" apartments had a central ventilation system with MERV filters, we speculate that equilibrium was reached where the release of culturable bacteria by indoor sources plus their penetration from outdoors was equal to or higher than removal of bacteria by the filtration system.

3.4.5 Outdoor Dew Point and Outdoor Temperature

The outdoor environmental parameters, such as outdoor DP and temperature, were compared during the four seasons and between the three buildings during each season. The outdoor DPs and temperatures stratified by the seasons and buildings are shown in Figure B-3a and Figure B-3b, respectively.

The effect of seasons without stratification by buildings

The overall outdoor DPs ranged from below 0 °F to 79 °F. As could be expected, it was significantly different during the four seasons (χ^2 (3) = 44.851, p < 0.001). The highest outdoor DPs were measured in summer, with a median of 62.59 °F (25th: 56.74 °F, 75th: 65.57 °F), and were significantly higher than the outdoor DPs for the other three seasons (p < 0.001). The lowest outdoor DPs were measured in winter (25th: 7.32 °F, median: 10.14 °F, 75th: 28.52 °F) and were significantly lower than the outdoor DPs for the other three seasons (p < 0.05). The outdoor DPs in fall, with a median of 32.99 °F (25th: 27.74 °F, 75th: 44.08 °F), were not different from the outdoor DPs in spring (25th: 28.62 °F, median: 39.28 °F, 75th: 60.10 °F; p = 0.434).

The outdoor temperatures ranged from 20.48 °F to 89.18 °F. Again, as could be expected, they significantly different among the four seasons (χ^2 (3) = 48.430, p < 0.001) and varied in a pattern analogous to outdoor DP. As expected for the Northeastern US, outdoor temperatures in summer (25th: 76.86 °F, median: 79.50 °F, 75th: 84.59 °F) were significantly higher (p < 0.05) than during other seasons; the outdoor temperatures were the lowest in winter (25th: 33.42 °F, median: 39.88 °F, 75th: 51.60 °F) with p < 0.05. Fall (25th: 47.42 °F, median: 53.01 °F, 75th: 60.10 °F) had similar outdoor temperatures to that of spring (25th: 51.23 °F, median: 60.61 °F, 75th: 69.50 °F).

Differences of outdoor parameters at three buildings during individual seasons

Spring was the only season that had significantly different outdoor DPs among the buildings (χ^2 (2) = 5.753, p = 0.028). The vicinity of B1 (median: 37.68 °F) had significantly lower outdoor DPs compared to near B3 (median: 62.53 °F; p < 0.001), while the vicinity of B2 (median: 60.10 °F) had comparable outdoor DPs close to B1 (p = 0.589) and B3 (p = 0.272). Lower outdoor DPs close to B1 could be explained by sampling in early spring compared to B3 that were sampled towards the end of the spring season. During summer, fall, and winter, the outdoor DPs were not different near the three buildings (p > 0.05).

The buildings were located within a 30-mile radius, so differences in outdoor temperature should be minimal; however, there could be effects of local structures, differences in sampled years, bodies of water, i.e., as near B2. The outdoor temperatures in spring, fall, and winter did not differ significantly among the buildings (p > 0.05), but they were different during summer (χ^2 (2) = 10.682, p = 0.005). Outdoor temperatures in summer close to B2 (median: 76.03 °F) were significantly lower than near B1 (median:

80.88 °F; p = 0.002); outdoor temperatures close to B3 were not different from those near to B1 (p = 0.276) and B2 (median: 79.93 °F) (p = 0.295). Lower outdoor temperatures near B2 could be explained by its proximity to the waterfront. Overall, we presumed that similar seasonal outdoor DPs and temperatures for the three buildings at different locations minimized any differences caused by them to indoor bioaerosol presence.

3.4.6 Indoor Dew Point

The survival and growth of bioaerosols are influenced by the presence of sufficient moisture in the air at favorable temperatures (Haas et al., 2014; Zheng Wang et al., 2001). Cellular stress increases in microorganisms when exposed to unsaturated air at high temperatures (Jensen & Schafer, 1998). While higher bioaerosol concentrations are often associated with high levels of relative humidity (RH) and temperature (Balasubramanian et al., 2012; DeKoster & Thorne, 1995; Horner, 2006; J.-H. Lee & Jo, 2006; Mentese et al., 2012; Moon et al., 2014; Pankhurst et al., 2011; Uk Lee et al., 2016; Wu et al., 2016), reliance on just RH and or just temperature is insufficient because taken separately they do not reflect the water content in the air, which is an important factor for microbial growth (Fabian et al., 2005). Hence, we used DP (°F) as an independent variable as it reflects water content in the air (Grinn-Gofroń et al., 2011; Jiřík et al., 2016; Ryan et al., 2002). The DP data were stratified by seasons and then by the buildings for each season (Figure 3-4). We considered the DP range between 45 °F to 55 °F to be a comfort range where residents did not experience dry or extremely humid conditions (CDC, 2018; Gaul & Underwood, 1952; News 8 Weather Blog, n.d.; NIOSH, 2016; NOAA, n.d.); in winter, the DP comfort range was lowered to 30 $^{\circ}$ F – 40 $^{\circ}$ F range to reflect the typical settings of HVAC systems. These comfort ranges were calculated based on the ASHRAE recommended indoor temperatures

(75 °F to 80.5 °F during summer; 68.5 °F to 75 °F during winter) (CDC, 2018; Ramspeck et al., n.d.) and US EPA recommended indoor relative humidity to reduce fungi growth (30 % to 60%) (CDC, 2018).

Spring: The indoor DPs differed significantly among the three buildings ($\chi^2(2) = 15.014$, p < 0.001; Figure 3-4a). B3 had a median indoor DP of 64.80 °F, and it was significantly higher than in the other two buildings (p < 0.001), and this median value was above the comfort zone. On the other hand, the median values of indoor DPs in B1 (median: 44.5 °F) and B2 (median: 54.35 °F) were almost within and in the comfort zone, respectively, although they differed significantly (p < 0.001). About 50% of DP values measured in B1 were outside the comfort zone.

Summer: The indoor DPs among the three buildings were significantly different ($\chi^2(2) = 24.597$, p < 0.001; Figure 3-4b). B1 had a median DP of 63.77°, and it was significantly higher than in the other two buildings (p < 0.05), with the median value being above the comfort zone. Median indoor DPs in B2 (median: 57.67 °F) that had dehumidification and B3 (median: 59.65 °F) were marginally above the comfort zone as well, and DPs in these two buildings were not different (p = 0.653).

Fall: The indoor DPs measured in B1 (median: 44.01 °F) and B2 (median: 51.99 °F) differed significantly (U = 679.0, p = 0.034; Figure 3-4c). The median value of indoor DPs in B2 was in the comfort range, while the median value of indoor DP in B1 was slightly below the comfort range.

Winter: The comfort zone was reduced to 30 °F to 40 °F during winter due to lower temperatures outdoors. Similar to fall, B1 and B2 had significantly different DPs (U =

473.50, p < 0.001; Figure 3-4d). The median value of indoor DP in B2 was 34.65 °F, and it was in the comfort range, while the median DP of 27.36 °F in B1 was below the comfort zone. It seems that the addition of moisture in winter in B2 maintained the indoor DP within the comfort range.

In summary, we found that the central ventilation system in the B2 building controlled the indoor DP in the range from 25 °F to 65°F throughout the year. However, the indoor DP in the "window AC" apartments ranged from 2 °F to 75°F. Extreme DP values could lead to health problems. Low levels of moisture can lead to skin irritation including chapping, burning, itchiness, nasal dryness and congestion, and eczema in extreme cases (Gaul & Underwood, 1952),(Reinikainen & Jaakkola, 2003). High levels of moisture lead to damp environments and have also been associated with upper respiratory symptoms, cough, wheeze and asthma prevalence (US EPA, 2013).

3.4.7 Association of Bioaerosol Concentrations with Dew Point

A non-parametric correlation analysis was performed to determine the association of indoor bioaerosols with DPs, and this result is shown in Figure 3-5. The indoor culturable fungi ($r_s = 0.543$; p < 0.001; Figure 3-5a) and bacteria ($r_s = 0.240$; p < 0.001; Figure 3-5b) concentrations were pooled together from the three buildings (n = 407 for each) and they had a moderate and weak, respectively, yet significant association with indoor DP. It could be explained by the increase in fungi and bacteria growth due to high moisture levels within confined spaces (Building Science Corporation, 2002).

In order to further analyze the association between culturable bioaerosol concentrations and DP, the data was stratified into two apartment groups according to the ventilation system types – "central HVAC" with n = 159 apartments (i.e., all from B2) and

"window AC" with n = 248 apartments (pooled data from B1 and B3 that have window ACs and natural ventilation) – and the data are shown in Figure 3-6.

For the "Central HVAC" apartments, there was a statistically significant and moderate positive association between indoor culturable fungi concentrations and indoor DPs ($r_s = 0.575$; p < 0.001; Figure 3-6a). It has been observed that mechanical ventilation in general could cause pressure imbalances that induce fungal growth in the building envelope and redistribute fungal material in the buildings due to leaks and moisture problems (Horner, 2006). The same association for the "window AC" apartments was positive, strong, and significant ($r_s = 0.657$; p < 0.001; Figure 3-6b).

The association between indoor culturable bacteria concentrations and DPs were also statistically significant though weaker compared to associations for fungi: for the "central HVAC" apartments with $r_s = 0.348$ (p < 0.001; Figure 3-6c), and for "window AC" apartments with $r_s = 0.190$ (p < 0.001; Figure 3-6d). Peccia et al. reported the shielding of bacteria from desiccation by the cellular water uptake at relative humidity values ranging from 20 % and 95% (Peccia et al., 2001). AC cooling coils in mechanically ventilated systems have been studied to increase bioaerosols by the presence of water condensation (Wu et al., 2016). We further stratified the association between indoor bioaerosols and indoor DPs by the four seasons for "central HVAC" and "window AC" apartments independently. These findings are described in Figures B-4 and B-5, and Appendix B-2.

In addition to the use of DP as our primary independent variable, we also explored an association between bioaerosol concentration and T and indoor RH. For the "central HVAC" apartments, the indoor culturable fungi concentrations had a weak and moderate yet significant association with indoor temperature ($r_s = 0.282$; p < 0.001) and indoor RH ($r_s = 0.584$; p < 0.001), respectively. The same association for the "window AC" apartments was moderate for indoor temperature ($r_s = 0.368$; p < 0.001) and strong for indoor RH ($r_s = 0.641$; p < 0.001). For indoor culturable bacteria concentrations, the "central HVAC" apartments had moderate and weak yet significant association with indoor temperature ($r_s = 0.303$; p < 0.001) and indoor RH ($r_s = 0.277$; p < 0.001), respectively. The concentrations in "window AC" apartments had a weak borderline significant association with indoor RH temperature ($r_s = 0.085$; p = 0.090), but weak and significant association with indoor RH ($r_s = 0.190$; p < 0.001). Overall, the association patterns between indoor bioaerosol concentrations and temperature and RH were similar to those with DP. However, the bioaerosol associations with DP were stronger for all stratification scenarios and required fewer analyses as the effects of both temperature and RH were taken into account.

We also stratified bioaerosol concentrations according to three levels of DPs: less than 45°F (n = 164), between 45°F and 55°F (n = 79), and above 65°F (n = 164) regardless of building type (Figure 3-7). The DP levels were selected to represent DP below, in, and above the comfort zone for residents, as mentioned previously. There was a significant difference between indoor culturable fungi as a function of DP: $\chi^2(2) = 99.865$, p < 0.001 for fungi; Figure 3-7a and $\chi^2(2) = 22.171$, p < 0.001 for bacteria; Figure 3-7b. A similar result was reported by Moon et al., where increases in humidity and temperature were associated with higher culturable fungi and bacteria concentrations in high-rise apartment buildings (Moon et al., 2014). We observed the highest culturable fungi concentrations in the highest DP stratum (25th: 47 CFU/m³, median: 94 CFU/m³, 75th: 194 CFU/m³), and these concentrations were significantly higher than in the middle DP stratum (25th: 29 CFU/m³, median: 51 CFU/m³, 75th: 99 CFU/m³; p = 0.007) and the lowest DP stratum (25th: 8 CFU/m³, median: 23 CFU/m³, 75th: 54 CFU/m³; p < 0.001). The fungi concentrations in the lower DP stratum were also significantly lower (p < 0.05) than the concentrations in the higher DP ranges. Concentrations of culturable bacteria in the lowest DP stratum had a median of 195 CFU/m³ (25th: 118 CFU/m³, 75th: 311 CFU/m³) and were significantly lower (p = 0.068) than in the highest DP stratum with a median concentration of 283 CFU/m³ (25th: 181 CFU/m³, 75th: 437 CFU/m³). The concentrations of bacteria at the middle DP stratum (25th: 142 CFU/m³, median: 208 CFU/m³, 75th: 378 CFU/m³) were not significantly different from the lowest and the highest DP strata with p = 0.263 and p = 0.488, respectively. The data suggest that higher DP levels indoors are predictive of higher concentrations of culturable bioaerosols, especially culturable fungi.

We also recognize that the indoor DP levels are significantly associated with the outdoor DP levels ($r_s = 0.627$; p < 0.001) and) and that outdoor DP can affect the presence of indoor bioaerosols. A positive relationship between outdoor DP and outdoor bioaerosols was reported by Jiřík et al., and the association was more pronounced for fungi compared to bacteria (Jiřík et al., 2016). A study by Ryan et al. reported that an increase in bioaerosol concentration indoors is likely associated with higher indoor moisture due to outdoor moisture intrusion. This study also reported the inadequacy of HVAC systems to control moisture (Ryan et al., 2002). Common outdoor species such as *Cladosporium* and *Alternaria* found indoors have been positively associated with outdoor DP (Grinn-Gofroń et al., 2011).

3.4.8 Variability of Culturable Bioaerosol Concentrations over Time

Seven apartments were sampled during all three sampling campaigns in Building 1. The indoor culturable fungi and their I/O ratios stratified by the four seasons and three

campaigns are shown in Figure 3-8a and Figure 3-8b, respectively. Within each apartment, there were large differences in concentrations between seasons and campaigns. The median CV values of culturable fungi concentrations and I/O ratios for the seven apartments were 71% and 65%, respectively. In general, lower culturable fungi concentrations were measured in winter compared to summer. The I/O ratio for culturable fungi was generally below 1 except in a few cases (8 out of 56 measurements).

Indoor culturable bacteria concentrations and their I/O ratios stratified by the four seasons and campaigns are presented in Figure 3-9a and Figure 3-9b, respectively. The indoor bacteria concentrations fluctuated across repeats, with a median CV value of 71%, when the campaigns and seasons were combined together. However, the culturable bacteria I/O ratios varied greatly season-to-season, with a CV value of 124%. Spring and summer seasons had higher I/O ratios of culturable bacteria compared to winter. However, I/O ratios in one of the apartments (Apt #6) had very low levels of data dispersion (CV: 43%). In addition to seasonal variations, the culturable bioaerosol concentrations in the seven repeated apartments could have differed due to different occupants. Information on whether the same tenant lived in the repeated apartments during the sampling period was unavailable.

3.4.9 Methodological Limitations

A culture-based measurement of bioaerosols including infectious microorganisms is frequently used owing to its ease of use and convenience (Griffiths et al., 1996; Heo et al., 2017; Lange et al., 1997; Mainelis, 2019). Concentrations and compositions of microbial exposure can be detected using selective media and specific incubation temperatures (Mainelis, 2019). However, it is well-known, and the authors recognize that this method does not detect the viable but not culturable (VBNC) bioaerosols (Blais-Lecours et al., 2015; P.-S. Chen & Li, 2005; Park et al., 2015). Even though culture-dependent techniques may underestimate exposures (Lindsley et al., 2017; Šantl-Temkiv et al., 2019) and long-term sampling is needed to understand the dynamics of indoor bioaerosols (Mainelis, 2019), there is a wealth of culturable data available, and that provides an easy comparison among studies.

Questionnaire data pertaining to the number of occupants, their common indoor behavior, including cleaning and cooking frequencies, and the presence of pets, would have been beneficial to investigate the effect of indoor sources. However, the questionnaire dataset was incomplete and we could only speculate about the contribution of these potential indoor sources.

Additionally, our study had a sample size of 48 unique apartments in three buildings with repeated investigations in 7 apartments. Since the bioaerosol dataset was part of separate IAQ studies, an uneven number of samples were collected in each building, and not all seasons were sampled in each building. This small building sample size and opportunistic dataset could have an effect on the investigated environmental and building variables. Our findings help develop a general understanding of the bioaerosol concentrations typical to buildings with the two types of ventilations systems and are not representative for all apartments and all types of ventilation systems. Future studies should focus on evaluating bioaerosol exposure in multiple buildings with different ventilation systems, and more importantly, in residential multi-apartment buildings.

3.5. Conclusions

This investigation provides insights into the concentrations and seasonal variability of culturable bioaerosols in three multi-apartment residential buildings with ventilation systems. Despite some methodological limitations of the study, we showed that the culturable bioaerosol concentrations and their I/O ratios differed significantly depending on a season and ventilation system in the apartments. The indoor culturable fungi seemed to have minimal indoor sources and their concentrations and I/O ratios were significantly lower in apartments with the central HVAC system. The indoor culturable bacteria apparently had a substantial contribution from indoor sources, regardless of the ventilation system type. The indoor DP was better controlled in apartments with central HVAC, and their occupants were not susceptible to extreme DP levels compared to apartments with window ACs. We also found that the indoor bioaerosol concentrations were positively and significantly associated with dew point. Our findings suggest that the reduction of indoor dew point would lessen occupants' exposure to bioaerosols. However, if the levels of DP are lowered too much, it may lead to discomfort of residents. The repeated investigations of several apartments over time indicated the extent to which the concentrations could vary within an apartment. Overall, our study provides an indication of typical bioaerosol levels in the investigated multi-apartment residential buildings, and future studies can be complemented with our findings to develop recommendations for IAQ and comfort of residents.

3.6 Acknowledgments

The US Department of Housing and Urban Development (HUD), NJLHH-0202-09, NJHHU0019-13, and the US National Science Foundation (NSF), CMS-0725503. The

funding sponsors had no role in the design of the study, the collection, analysis, or interpretation of data, in the writing of the manuscript, and in the decision to publish these results. The authors would like to express their appreciation to the building managers for facilitating access to the study buildings and to the participants who graciously allowed us to access their apartments.

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Figure 3-1. Study timeline. Measurements were performed from 2011 to 2015 and measurement campaigns are stratified by buildings (B1, B2, and B3) and seasons (Sp: Spring, Su: Summer, F: Fall, and W: Winter). B1 was sampled thrice and referenced in this study as three campaigns (B1-C1, B1-C2, and B1-C3).

Table 3-1. Bui	ldings descriptions.	

Building	Building 1 (B1)	Building 2 (B2)	Building 3 (B3)
Designation	Energy Star built in 2009	Built in 2003; Leadership in Energy and Environmental Design (LEED) – Existing Buildings (EB) platinum in 2009	Built in 1926; retrofitted in 2006
No. of floors	6 and 7 (two wings)	27 floors	10 floors
Building type and Annual Income	Economy and \leq \$20,000	Luxury and \geq \$200,000	Economy and \leq \$20,000
Ventilation system	Natural ventilation	100% conditioned outdoor air	Natural ventilation
Filtration	No central filtration	MERV 7 and 14 on air intakes MERV 11 in fan coil units	No central filtration
Heating	Baseboard heating	Fan coil units	Baseboard heating
Air Conditioning	Window units	Central	Window units

Building code	Buil (B1)	lding)	1		Bui Car (B1	lding npaig -C1)	1 – 3n 1		Bui Can (B1-	lding npaig -C2)	1 – gn 2	1 -Building 1 -n 2Campaign 3(B1-C3)		Building 2 (B2)				Building 3 (B3)						
	Tota	ıl	-		Tote	Total			Total			Total				Total			Total					
Variables	Sp	Su	F	W	Sp	Su	F	W	Sp	Su	F	W	Sp	Su	F	W	Sp	Su	F	W	Sp	Su	F	W
Culturable Fungi	230				55				153				22				159				20			
and its I/O ratio	64	62	57	47	17	21	17		41	41	40	31	6			16	40	53	31	35	5	15		
Outdoor	56				26				24				6				22				8			
Culturable Fungi	17	16	14	9	9	9	8		6	7	6	5	2			4	5	8	4	5	2	6		
Culturable	230				55				153				22				159				20			
I/O ratio	64	62	57	47	17	21	17		41	41	40	31	6			16	40	53	31	35	5	15		
Outdoor	56				26				24				6				22				8			
Bacteria	17	16	14	9	9	9	8		6	7	6	5	2			4	5	8	4	5	2	6		
Indoor	228				55				151				22				159				20			
Temperature and Dew point	64	61	57	46	17	21	17		41	40	40	30	6			16	40	53	31	35	5	15		
Outdoor	46				26				15				6				19				8			
Dew point	14	15	12	6	9	9	8		3	6	4	2	2			4	5	7	4	3	2	6		

and B3) including campaigns of B1 (B1-C1, B1-C2, and B1-C3) and seasons (Spring – Sp; Summer – Su; Fall – F; Winter – W).

Table 3-2. Number of samples collected in multi-residential apartments and its outdoors for variables stratified by buildings (B1, B2,





a)



Figure 3-2. a) indoor culturable fungi concentration (CFU/m³) and b) culturable fungi indoor-outdoor ratio stratified by the buildings (B1, B2, and B3) and four seasons (Spring, Summer, Fall, and Winter). The dotted red line represents the indoor/outdoor ratio equal to 1. The upward-facing triangles and downward-facing triangles represent 1st and 99th percentile of the data, respectively; the whiskers represent 1.5x of interquartile range; the square represents the mean; the lower, middle and upper lines in the box plot are 25th percentile, median, and 75th percentile of the data, respectively. * and # represent group(s) that are significantly different with a p-value less than 0.05 and 0.1, respectively.

Table 3-3. Culturable fungi concentrations (CFU/m³) and its indoor/outdoor (I/O) ratios for the study buildings (B1, B2, and B3) and campaigns of Building 1 (B1-C1, B1-C2, and B1-C3) stratified by seasons (Spring – Sp; Summer – Su; Fall – F; Winter – W). The overall concentrations are values without seasonal stratifications.

Variable	Overall	Spring (Sp)	Summer (Su)	Fall (F)	Winter (W)	
	Median (25 th – 75 th)	Median (25 th - 75 th)	Median (25 th - 75 th)	Median (25 th – 75 th)	Median (25 th - 75 th)	
Culturable fungi (CFU/m ³)						
B1	71 (32 – 146)	58 (29 – 204)	111 (75 – 215)	64 (42 - 110)	21 (12 - 61)	
B1-C1	95 (51 – 167)	60 (35 - 83)	142 (79 - 300)	105 (64 - 123)		
B1-C2	72 (32 – 159)	78 (32 – 229)	110 (75 - 200)	57 (40 - 106)	18 (6 - 54)	
B1-C3	31 (21 – 53)	29 (24 – 35)			32 (20 - 66)	
B2	27 (10 – 52)	19 (11 – 51)	36 (28 – 57)	43 (18 – 79)	5 (3 – 10)	
B3	156 (78 – 241)	149 (65 – 236)	162 (96 – 241)			
Culturable fungi I/O						
B1	0.53 (0.34 – 0.85)	0.52 (0.36 – 0.78)	0.62 (0.45 – 0.86)	0.50 (0.36 – 0.83)	0.35 (0.14 - 0.96)	
B1-C1	0.54 (0.26 - 0.75)	0.32 (0.16 – 0.53)	0.61 (0.28 - 0.86)	0.60 (0.45 - 0.88)		
B1-C2	0.53 (0.36 - 0.85)	0.54 (0.39 - 0.81)	0.62 (0.54 - 0.85)	0.46 (0.36 - 0.80)	0.35 (0.14 - 1.39)	
B1-C3	0.46 (0.18 - 1.02)	0.90 (0.44 - 1.13)			0.38 (0.15 - 0.83)	
B2	0.18 (0.10 - 0.36)	0.20 (0.10 - 0.36)	0.16 (0.12 – 0.29)	0.13 (0.07 – 0.41)	0.27 (0.11 – 0.56)	
B3	0.61 (0.49 – 0.79)	0.56 (0.52 – 0.78)	0.63 (0.42 – 0.75)			







Figure 3-3. a) indoor culturable bacteria concentration (CFU/m³) and b) culturable bacteria indoor-outdoor ratio stratified by the buildings (B1, B2, and B3) and by four seasons (Spring, Summer, Fall, and Winter). The dotted red line represents the indoor/outdoor ratio equal to 1. The upward-facing triangles and downward facing triangles represent 1st and 99th percentile of the data, respectively; the whiskers represent 1.5x of interquartile range; the square represents the mean; the lower, middle and upper lines of the box plot are 25th percentile, median, and 75th percentile of the data, respectively. * represents a group that is significantly different from others with a p-value less than 0.05.

Table 3-4. Culturable bacteria concentrations (CFU/m³) and its indoor/outdoor (I/O) ratios for the study buildings (B1, B2, and B3) and campaigns of Building 1 (B1-C1, B1-C2, and B1-C3) stratified by seasons (Spring – Sp; Summer – Su; Fall – F; Winter – W). The overall concentrations are values without seasonal stratifications.

Variable	Overall	Spring (Sp)	Summer (Su)	Fall (F)	Winter (W)		
	$Median~(25^{th}-75^{th})$	$Median~(25^{th}-75^{th})$	$Median~(25^{th}-75^{th})$	Median (25 th – 75 th)	$Median~(25^{th}-75^{th})$		
Culturable Bacteria (CFU/m ³)							
B1	235 (153 - 404)	241 (148 - 461)	278 (180 - 437)	200 (150 - 294)	235 (130 - 377)		
B1-C1	266 (154 - 606)	235 (133 - 388)	437 (222 – 789)	196 (154 – 470)			
B1-C2	226 (153 - 370)	249 (173 - 484)	226 (180 - 330)	220 (148 - 272)	210 (116 - 368)		
B1-C3	279 (144 - 380)	194 (143 – 376)			290 (195 - 414)		
B2	219 (133 – 326)	183 (136 – 362)	276 (187 – 447)	225 (172 - 377)	135 (82 – 179)		
B3	214 (111 – 331)	169 (106 – 389)	229 (136 - 311)				
Culturable Bacteria I/O							
B1	1.13 (0.63 – 2.07)	1.03 (0.38 - 2.09)	1.42 (0.92 – 2.63)	1.15 (0.73 – 1.89)	0.82 (0.40 - 1.36)		
B1-C1	1.32 (0.69 – 3.27)	1.13 (0.64 – 1.91)	2.27 (1.26 - 8.25)	0.85 (0.61 - 1.69)			
B1-C2	1.03 (0.60 - 1.74)	0.99 (0.38 - 1.83)	1.28 (0.85 - 1.88)	1.27 (0.85 – 1.98)	0.76 (0.35 – 1.29)		
DGB1-C3	1.31 (0.46 - 2.30)	1.83 (0.33 - 3.55)			1.18 (0.47 – 1.96)		
B2	1.38 (0.57 – 2.91)	0.76 (0.37 -1.86)	1.47 (0.68 – 3.23)	2.39 (1.56 - 3.78)	1.15 (0.51 – 3.76)		
B3	1.16 (0.70 – 2.29)	1.28 (0.81 – 3.06)	1.08 (0.70 - 1.98)				

a)

SPRING



d)

SUMMER













Figure 3-4. Indoor dew point (° F) for the four seasons(a: Spring, b: Summer, c: Fall, and d: Winter) stratified by the three buildings (B1, B2, and B3). The dotted blue lines represent the zone of 45-55 ° F. The upward-facing triangles and downward-facing triangles represent 1^{st} and 99^{th} percentile of the data, respectively; the whiskers represent 1.5x of interquartile range; the square represents the mean; the lower, middle and upper lines of the box plot are 25^{th} percentile, median, and 75^{th} percentile of the data, respectively. #, *, and ** represent group(s) that are significantly different with p-value less than 0.1, 0.05, and 0.001, respectively.



Figure 3-5. Association of a) indoor culturable fungi concentrations (CFU/m³) and b) indoor culturable bacteria concentrations (CFU/m³) with indoor dew points (°F). Correlation analysis was given by Spearman correlation coefficient (r_s) along with the *p*-value; *n* represents the number of samples; the trendline in red and the equation represents the line of best fit.



Figure 3-6. Association indoor culturable fungi concentrations (CFU/m³) (a and b) and indoor culturable bacteria concentrations (CFU/m³) (c and d) with indoor dew point (°F) stratified by the type of ventilation system: central Heating Ventilating and Cooling system ("central HVAC" apartments; a and c) and with radiator heat and window air-conditioning (AC) units ("window AC" apartments; b and d). Correlation analysis was given by Spearman correlation coefficient (r_s) along with the p-value; n represents the number of samples for each season; the trendline in red and the equation represents the line of best fit.



a)

b)

Figure 3-7. Indoor bioaerosol concentrations stratified by three levels of dew point: dew point of less than $45^{\circ}F$ shown in green, between $45^{\circ}F$ and $55^{\circ}F$ shown in yellow, and above $55^{\circ}F$ shown in red. The data from all buildings and seasons are combined. a) Indoor culturable fungi concentrations (CFU/m³) b) Indoor culturable bacteria concentrations (CFU/m³). The upward-facing triangles and downward-facing triangles represent 1st and 99th percentile of the data, respectively; the whiskers represent 1.5x of interquartile range; the square represents the mean; the lower, middle and upper lines of the box plot are 25th percentile, median, and 75th percentile of the data, respectively. **, *, and # represent group(s) that are significantly different with a p-value less than 0.001, 0.05 and 0.1, respectively.



b)



Figure 3-8. a) indoor culturable fungi concentrations (CFU/m³) and b) culturable fungi indoor-outdoor ratio stratified by seasons (Sp: Spring, Su: Summer, F: Fall, and W: Winter) and sampling campaigns (B1-C1, B1-C2, B1-C3) for seven apartments that were repeatedly sampled in Building 1. The dotted blue line represents the ratio at which indoor concentrations are equal to outdoor concentrations. Measurements of C1 are in black, C2 are in light grey, and C3 are in red.





b)

a)

Figure 3-9. a) indoor culturable bacteria concentrations (CFU/m³) and b) culturable bacteria indoor-outdoor ratio stratified by seasons (Sp: Spring, Su: Summer, F: Fall, and W: Winter) and sampling campaigns (B1-C1, B1-C2, B1-C3) for seven apartments that were repeatedly sampled in Building 1. The dotted blue line represents the ratio at which indoor concentrations are equal to outdoor concentrations. Measurements of C1 are in black, C2 are in light grey, and C3 are in red.

CHAPTER 4. EVALUATION OF PERSONAL EXPOSURES TO BIOAEROSOLS USING FIVE DIFFERENT PERSONAL SAMPLERS IN SEVERAL ENVIRONMENTS

4.1 Abstract

The information on the biological performance of personal bioaerosol samplers – as well as characterization of the captured particles in terms of their size distribution, viability, and culturability status – is limited. Here, we used five commercially available or recently developed personal (bio)aerosol samplers to study personal exposures to bioaerosols at three different locations and compared sampler performance. The used samplers were: Personal Electrostatic Bioaerosol Sampler (PEBS) developed at Rutgers University, CIP 10-M, Ultrasonic Personal Aerosol Sampler (UPAS), NIOSH Personal Bioaerosol Cyclone Sampler 251 (NIOSH), and Button Aerosol Sampler. The personal bioaerosol exposures were evaluated in terms of total concentrations, adenosine triphosphate (ATP) concentrations, and culturable concentrations of bacteria and fungi. Viable, dead, and injured fractions of captured particles were also compared. Two sets of samplers were placed on two mannequins to minimize experimental variability, and a total of 96 samples were collected in a horse farm, greenhouse and outdoors.

We found that the personal bioaerosol exposures varied significantly between the sites (p < 0.05), and intra-variability between the two sets of each sampler was not statistically significant (p > 0.05). The NIOSH sampler and filter-based samplers (Button and UPAS samplers) measured the highest total bioaerosol concentrations (bacteria: 10^6 #/m³; fungi: 10^5 #/m³), while samples captured by PEBS had the highest live cell fractions (37.5 ± 8.4%; p < 0.05). The personal samplers, except CIP 10-M sampler, measured

similar culturable bacteria concentrations (up to $5 \times 10^3 \text{ CFU/m}^3$), whereas the culturable fungi proportions were similar between the five samplers (p > 0.05). We conclude that a choice of a personal bioaerosol sampler, as well as its interaction with the exposure measurement metric, would likely affect the determined bioaerosol exposures. The obtained sampler performance data and insights into their use provide information that would be useful for further sampler developments and also when choosing tools for personal bioaerosol exposure assessments.

Keywords

Bioaerosols - Exposure assessment - Personal samplers - Personal bioaerosol exposure -Viability - Total and culturable bioaerosol concentrations

4.2 Introduction

Exposure to airborne particulate matter (PM) is known to cause negative health effects, including exacerbation of respiratory tract diseases (Falkinham, 2003; Xu et al., 2018), cardiovascular diseases (Dabass et al., 2018; Dominici et al., 2006; Erqou et al., 2018), mucous irritation and infections in immunocompromised persons and general population (Hansen et al., 2012; Pankhurst et al., 2011; Tageldin et al., 2017), and early mortality (Klemm & Mason, 2000; Schwartz et al., 1996; US EPA, 2014; Q. Wang et al., 2018). Furthermore, the International Agency for Research on Cancer (IARC) has classified airborne particulate matter (PM) from outdoor air pollution as carcinogenic to humans (IARC Group 1) (Loomis et al., 2014). One of the components of PM is bioaerosols, e.g., PM that is biological in origin, such as viruses, bacteria, fungal spores, fragments of fungi mycelium, pollens, and their by-products (toxins)(Cox & Wathes, 1995; Fröhlich-Nowoisky et al., 2016). The ubiquitous and variable presence of bioaerosols, their negative

health effects as well as their role in environmental processes make it imperative to monitor and characterize bioaerosols, including their concentration, size distribution, species, viability, and culturability status.

The development of novel bioaerosol sampling tools is one of the grand aerosol challenges, as discussed at the International Aerosol Conference 2018 (St. Louis, MO) (Mainelis, 2019; Sorensen et al., 2019). In particular, there is a crucial need to identify and develop sampling tools that would improve the representative measurement of bioaerosols and help us better understand the connection between personal exposures to bioaerosols and health outcomes. Current bioaerosol monitoring practices at indoor and outdoor locations typically utilize stationary and portable bioaerosol samplers. These samplers are used to estimate bioaerosol exposure levels for workers in industrial, agricultural, and animal farming environments as well as occupants in residential and office buildings (Quinlan & Plog, 2012). However, most of such samplers are prone to under- or overestimating personal exposures (Lindsley et al., 2017; Mainelis, 2019; Sagona et al., 2015; Z. Wang et al., 2012). Hence, personal exposures to bioaerosols in various environments should be characterized using *personal* bioaerosol samplers (Mainelis, 2019; C.-H. Wang et al., 2015).

Although several personal bioaerosol samplers have been developed or adapted for bioaerosol sampling in the last two decades, comparative studies have typically focused on their physical performance, and there are currently limited field studies focused on the biological performance of recently developed personal bioaerosol samplers. Studies examined personal sampling using impingers (Duchaine et al., 2001; Zheng & Yao, 2017), rotating cups (Görner et al., 2006), filters (V. Aizenberg et al., 2000), micro-centrifuge tubes (Su et al., 2012) and, recently, electrostatics-based collection (T. Han & Mainelis, 2008; T. T. Han et al., 2017, 2018). For this study, we chose five personal samplers that were commercially available or recently developed and compared their relative biological performances in terms of determined bioaerosol number concentrations and viability and culturability of captured biological particles. To simplify the complex and challenging aspects of the bioaerosols study field, the term "bioaerosol(s)" refers here to bacteria and fungi communities exclusively.

The five personal samplers were: Personal Electrostatic Bioaerosol Sampler (PEBS) developed at Rutgers (T. T. Han et al., 2017, 2018), CIP 10-M sampler (CIP 10-M; Air Sampling Devices, Milford, NH), Ultrasonic Personal Aerosol Sampler (UPAS; Access Sensor Technologies, Fort Collins, CO), Personal Bioaerosol Cyclone Sampler 251 (NIOSH) developed by the National Institute for Occupational Safety and Health (NIOSH, Morgantown, WV) and Button Aerosol Sampler (Button; SKC, Eighty Four, PA, USA). All samplers except UPAS have been either designed (E.g., PEBS, CIP, and NIOSH 251) or adapted (e.g., Button) for bioaerosol sampling. UPAS has been designed for measuring personal exposures to PM_{2.5}; however, it is a compact sampler that could be adapted to sample bioaerosols and included in our study as a first such attempt. The technical characteristics of the samplers are described in the Methods section. These samplers had different flow rates (from 1 to 10 L/m), the fraction of particles collected, i.e., inhalable/respirable/PM2.5 fractions, collection mechanisms (centrifugal, electrostatic, filtration) and collection mediums (filter, liquid, dry tubes). The proportions of live and dead cells in ambient and indoor environments for these five samplers have also not been previously reported.

The primary objectives of the study were to answer the following questions:

- How does the choice of a personal sampler affect the determined exposures to fungi and bacteria concentrations?
- 2) How do personal samplers affect the viability and culturability of collected samples?
- 3) What is the effect of sites on personal bioaerosol exposures?
- 4) What is the intra-variability of samplers in terms of their biological performance?

Additionally, we were also interested in better understanding the potential advantages and disadvantages of samplers during field use, including their user-friendliness and limits of their application.

4.3 Methodology

4.3.1 Sampling Design

A set of five personal samplers was placed each on two mannequins (M1 and M2) in their chest region to measure personal bioaerosol exposures. The mannequins were a substitute due to the practical limitations of simultaneously testing five personal samplers along with their pumps on people and eliminated any sampling biases contributed by human presence. All samplers were operated simultaneously for 4 hours to represent a half of a typical work shift duration, between 10 am and 2 pm, and the testing was repeated at each site for two days. The sampling time was kept constant to minimize plausible temporal variations of bioaerosols (Cho et al., 2006; Lighthart, 2000). Sampling was performed at three sites, and a total of 12 samples per sampler type were collected. The samplers of each type were randomly assigned the mannequins M1 and M2, and the assignment was kept consistent

during the entire study. A total of 96 samples were collected with the five samplers at three sampling sites. The samplers were transported individually in clean, new re-sealable bags (Ziploc, Johnson & Son Inc.) for each sampling day and attached to the mannequins using 2-inch-wide snap-together fasteners (McMaster Carr Inc., Chicago, IL).

4.3.2 Description of the Sampling Sites

Samples were collected at three distinct locations on Rutgers Cook Campus, New Brunswick, NJ, from November to December of 2018. The sampling sites were chosen to subject the samplers to varied meteorological factors and bioaerosol sources. These factors have been reported to influence biological abundance (Šantl-Temkiv et al., 2019; Zhu et al., 2003). The locations were a horse barn (Site 1) representing an agricultural environment, outdoor (Site 2) by the Environmental Science and Resources (ENR) building representing a mixed rural and town environment, and the greenhouse (Site 3) representing a controlled indoor environment. At Site 1 (Figure 4-1a), the samples were collected in an empty semi-open horse stall, while the adjacent stalls were occupied by horses. The stalls had dimensions of 4 m x 4 m, and their floor was covered with hay. The mannequins were placed in the middle of the empty stall. Barn doors were kept closed during sampling except during entry and exit. The mean temperature (t_m) was 9 ± 3 °C, and the mean relative humidity (RH_m) was 35 ± 1 % during sampling. Site 2 (Figure 4-1b) was an open-air location bordered by natural vegetation with an animal farm, Rutgers organic garden about ~50 m away, and the ENR building on the adjacent side. The mannequins were located approximately in the middle between the two structures, about ~15 m from each one of them, and t_m of 3.1 ± 2 °C and RH_m of 35 ± 5 % were recorded at Site 2 during sampling. Site 3 was a room with dimensions 50 m x 50 m in a greenhouse with a t_m of 22.5 ± 2 °C and an RH_m of 70 ± 1 % (Figure 4-1c). The sampled room was selected to represent summer-like temperature and RH. The room was used to grow basil plants in pots placed on tables and elevated at 1m from the floor. The mannequins were placed approximately in the middle of the room. These sites differed by wind speeds, temperature, relative humidity, and local microenvironments, e.g., the greenhouse room had basil plants that were watered daily, and stable floors were layered with hay.

4.3.3 Sampler Description and Setup

The four commercially available personal aerosol and bioaerosol samplers and one personal bioaerosol sampler built in-house used in the study were PEBS, CIP 10-M, UPAS, NIOSH, and Button. Specifications of these samplers are provided in Table 4-1.

4.3.3.1 Field-deployable Personal Electrostatic Bioaerosol Sampler (PEBS)

PEBS, a personal sampler concept developed at Rutgers (T. T. Han et al., 2017, 2018), features a two-stage electrostatic precipitator and operates at a relatively high flow rate of 10 L/m. The current version of the PEBS is shown in Figure 4-2c. It consists of a core part (Figure 4-2a) and an external box with compact power supplies (Figure 4-2b). The sampler's core part was previously tested with both PSL and biological particles in the laboratory and yielded a collection efficiency of 75% (T. T. Han et al., 2017). This field study provided an opportunity to examine the performance of the sampler's core part as well as the suitability of the electronic components in the field before they are incorporated into a single unit.

The core part of the PEBS (Figure 4-2a) consists of four components: a sharp-edged inlet, a wire-to-wire charger creating low ozone emission (less than 10 ppb), a collector having a removable dual-side metal plate and two stainless steel quarter-cylinder grounded

electrodes, and an air mover (e.g., a fan with a protective grill). It has a shape of a cylinder of 2.54 cm (1 inch) in diameter and ~14 cm (6 inches) in length. A sharp-edged inlet fabricated by 3D printing and an inlet screen (nickel electroformed screen: 20×20 mesh, 65 µm in diameter, 90% fraction of open area, Industrial Netting Inc., Minneapolis, MN) are attached to the inlet to prevent unwanted large-sized debris such as insects, plant fragments, and fibers from entering the sampler. The dual-sided collection plate was coated by hydrophobic surface (HIREC-1450, NTT Corporation Inc., Japan) for efficient particle removal.

For this study, all components necessary to operate this field-deployable PEBS, including DC-to-DC high voltage power converter/supply (EMCO Corp., Sutter Creek, CA), batteries (e.g., 3.7 V/1200 mAh rechargeable lithium), voltage regulators (QS-1212CCBA-80W, Qskjpower Co., China), voltage meter (130A Watt, Powerwerx Inc., Yorba Linda, CA), and switches (Grainger Inc., South Plainfield, NJ) were placed in a control box with dimensions 152 mm \times 152 mm \times 95 mm (Figure 4-2b). The voltages of the power supplies, as well as the fan's flowrate, were adjusted via controls on the front of the box (Figure 4-2b).

4.3.3.2 CIP 10-M sampler for Microorganisms (CIP 10-M sampler)

CIP 10-M sampler is designed for personal bioaerosol sampling and uses a liquid-filled cup rotating at approximately 7000 rpm resulting in a sampling flow rate of 10 L/min (Görner et al., 2006). The direct sampling into liquid medium should minimize stress to bioaerosols; however, the sampling duration is limited due to the high evaporation rate of the rotating collection fluid (Duquenne et al., 2012; Simon et al., 2016). An inhalable inlet was selected to capture particle size ranges similar to that of other samplers. The rotating

cups were washed and autoclaved in an autoclavable bag (Fisher Scientific, Pittsburg, PA), and loaded on to the body of the sampler in the laminar flow chamber (NuAire Class II, Type A2, Plymouth, MN, USA). CIP 10-M unit was wiped dry with 70% ethanol (Volu Sol, Fisher Scientific Inc.) using Kim Wipes (Kimberly-Clark Professional, Roswell, USA) to prevent cross-contamination between sites. Before sampling start, the inhalable head was removed, and 2 mL of phosphate-buffered saline (PBS) solution (cat no.: P4417; Sigma-Aldrich Inc.) was added into the cup in the upright position. The sampler was started and then placed in a holder that was firmly attached to a mannequin. The rotational speed was periodically checked using the pocket laser tachometer (model PLT 200; Monarch Instrument Inc.). To compensate for liquid evaporation, the sampler was switched off every 60–90 mins and 1 mL of PBS solution (Sigma-Aldrich Inc.) was added on site.

4.3.3.3 Ultrasonic Personal Aerosol Sampler (UPAS)

UPAS is a lightweight filter sampler with a cyclone inlet designed to collect PM_{2.5} (Volckens et al., 2017). UPAS has been previously used in estimating PM_{2.5} exposures for household air pollution (Arku et al., 2018) and rural communities (Pillarisetti et al., 2019). In our study, a 37 mm PTFE filter with 2 µm pore size (SKC Inc.) was placed in the filter cartridge. UPAS was recharged and cleaned prior to placing it on the mannequins. Manufacturer's protocols for cleaning and operating of UPAS were followed. In short, the cyclone inlet cap was sprayed with 70% ethanol (Fisher Scientific Inc.) and wiped dry with Kim Wipes (Kimberly-Clark Professional Inc.). The outer cyclone inlet cap was layered with a high vacuum silicone grease (Acros Organics, Geel, Belgium) to prevent the dislodging of the PM trapped on cyclone wall surfaces. The top and bottom halves of the filter cartridge were separated using its tabs, cleaned with 70% ethanol (Fisher Scientific

Inc.) followed by loading of the filter using autoclaved forceps. A sampling duration of four hours was pre-set via an app. The flow rate was verified at the start of each sampling day using a flow-measurement adapter and the mass flow meter (Model 4040 F; TSI Inc.).

4.3.3.4 NIOSH Personal Bioaerosol Cyclone Sampler 251 (NIOSH sampler)

The NIOSH sampler features two stages and a back-up filter that collects size-fractionated air samples by centrifugal force (Lindsley et al., 2006; NIOSH, 2017). Particles larger than 4 µm are captured in the 1st stage (referred here as NIOSH-L, where L stands for large particles), with a 50% cut-off particle size of 4.1 µm. The 2nd collection stage (referred here as NIOSH-S stage, where S stands for small particles) captures particles between 1 and 4 μ m (50% cut-off particle size of 1 μ m). The remaining particles, i.e., those < 1 μ m, are collected on the back-filter (referred here as NIOSH-F, where F stands for filter stage). The sampler is operated at 3.5 L/min provided by an external pump. The NIOSH sampler has been previously used for the size-fractionated collection of fungal spores and fragments in ambient environments (Lindsey et al., 2006) and airborne bacteria at a duck production facility (Martin et al. 2015). We followed instructions for assembly, calibration, and cleaning of the sampler provided by NIOSH (2017). In short, the assembled filter cassette (37mm, SKC Inc.) with the filter (37mm, 2µm pore size PTFE filter, SKC Inc.) and support pad (37mm, SKC Inc.) was firmly placed on the top of the sampler. NIOSH-L and NIOSH-S stages were connected with polystyrene centrifuge tubes of 15 mL and 1.5 mL volume, respectively. An external pump (SKC Inc.) set to sample for four hours was connected to the outlet of the filter cassette. The sampler's flow was calibrated using a custom flow calibration adapter provided by NIOSH and a flow meter (TSI mass flow meter, TSI Inc.). For cleaning, NIOSH samplers were washed in soapy water, sprayed down with 70%

ethanol (Fisher Scientific Inc.), and then left to dry overnight before use. An electric duster (ED 500 Data Vac, Metro Vacuum, USA) was used to dry the samplers and remove residue, if any. The three stages of the sampler (L, S, and F) were analyzed separately since they provide information about specific biological particle size fractions and viability and culturability for the fractions. To report the total, ATP, and culturable bioaerosol concentrations, we summed up the yield by the NIOSH-L, NIOSH-S, and NIOSH-F stages, and refer to the sum as that provided by NIOSH-T sampler (T stands for total) for each repeat.

4.3.3.5 Button Aerosol Sampler (Button sampler)

The button sampler is a commonly used filter sampler for studying personal inhalation exposure. It has a dome-shaped porous sampling inlet that has low sensitivity to wind velocity and direction (Vitaly Aizenberg et al., 1998; V. Aizenberg et al., 2000; Adhikari et al., 2003; Zheng Wang et al., 2001; T. Han et al., 2015; Lee et al., 2006). It was operated with a 25mm PTFE filter (0.45 µm pore size, SKC Inc.), and the sampling flow rate of 4 L/min was provided by a fully charged, calibrated pump (AirChek XR500, SKC Inc.). The sampler was cleaned by soaking it in 70% ethanol (Fisher Scientific Inc.) and wiped dry with Kim Wipes (Kimberly-Clark Professional Inc.). On the day of sampling, new PTFE filters (SKC Inc.) were loaded using autoclaved forceps in the laminar flow chamber (NuAire, Inc.), and the sampler was attached to the mannequins at the sampling sites.

4.3.4 Sample Retrieval and Elution

At the end of each sampling day, all samplers except liquid samples of CIP 10-M were transported in a carrier (DEWALT Storage Unit) within marked plastic re-sealable bags (Johnson & Son Inc.) and disassembled in the laminar flow chamber (NuAire, Inc.). The
following procedures were followed for sample retrieval. The samples from each sampler were either eluted or reconstituted to 5mL of PBS (Sigma-Aldrich Inc.) and then subdivided for different analyses.

<u>PEBS</u>: The charging and collection sections were separated by unlocking the twist-lock fastener, and the collection plate was removed. The plate was transferred into a 15mL autoclaved jar filled with 5 mL of PBS (Sigma-Aldrich Inc.). The hydrophobic coating allowed for easy elution of collected particles by vortexing the jar for 1 minute.

<u>*CIP 10-M*</u>: The sampler was switched off, and the inhalable inlet was removed at the site in the upright position to prevent any spillage. The collection liquid was immediately transferred into a sterile tube. The cup was further rinsed with 1 mL of PBS (Sigma-Aldrich Inc.) in the laminar flow chamber (NuAire, Inc.) and then added to the sample previously collected onsite.

<u>UPAS</u>: The cyclone inlet cap was unscrewed, and the filter was placed in a 50 mL sterile conical tubes (Falcon, Fisher Scientific Inc.) filled with 5 mL of PBS solution (Sigma-Aldrich Inc.). The sample was extracted from the filter by vortexing for 2 mins, followed by ultrasonic agitation (Branson 8800 Series Ultrasonic Cleaner, Richmond, VA) for 15 mins.

<u>*NIOSH:*</u> 5 mL of PBS solution (Sigma-Aldrich Inc.) was added directly into NIOSH-L, and 1 mL was added into NIOSH-S, followed by vortexing for 20-30 sec. NIOSH-S sample was transferred into a 15 mL tube, and an additional 1 mL was added into the original tube followed by vortexing for 20-30 sec. For the filter (NIOSH-F) section of the NIOSH sampler, the same elution procedure as for UPAS was used.

<u>Button</u>: The sampler's inlet was removed, and the filter was gently placed in a 50 mL sterile conical tube (Fisher Scientific Inc.) filled with 5 mL of PBS solution (Sigma-Aldrich Inc.) using autoclavable forceps. The sample was collected using the extraction procedure mentioned previously for UPAS.

4.3.5 Methods used to Determine Total Particle Concentration, Viability, and Culturability

4.3.5.1 Microscopy

The number of collected bacteria was determined using acridine orange epifluorescence microscopy (AOEM) with the Axioskop 20 (Carl Zeiss MicroImaging Inc., Thornwood, NY) and all samples were counted in triplicate. This method has been described previously by Therkorn et al. (2017). Additionally, we modified the staining procedure to improve the uptake of stain. Here, 100µL of each sample was treated with 10µL formaldehyde (37% by weight, Fisher Scientific, 1 Reagent Lane, Fairlawn, NJ), vortexed for 30 secs, and left undisturbed for 15 mins. Then, 890µL of 1x acridine orange (AO) solution (Becton Dickinson Microbiology System, Sparks, MD) was added and treated by water steam for 45 mins with samples kept in the dark. The samples were counted under 100X. The resulting total airborne concentration of bacteria ($C_{bacteria}$ (#/m³)) was calculated as follows:

$$C_{bacteria} (\#/m^3) = \frac{N \times M \times D \times V_s \times 1000}{Q \times t}(Eq. 1)$$

where N is the average cell count per microscope view field; M is the number of view fields for a 25-mm filter (M =6125); D is the dilution factor; V_s is the entire sample volume, mL; Q is the flowrate in Liters/min; t is the sampling time in min. The total number of airborne fungal spores was using direct light microscopy and a hemocytometer chamber (model 3200; Hausser Scientific, Horsham, PA). The chamber was carefully cleaned, and a coverslip covered the counting grid. The sample was vortexed for 15 sec, and 10 μ L was gradually loaded under the coverslip. The spores were counted under a 40X lens, and each sample analyzed in triplicate. The total fungi spore concentration in each sample, C_{fungi} (#/m³), was using Eq. 1, except the value of M was 10,000 when counting four corner squares plus a middle square of the chamber.

4.3.5.2 ATP based Bioluminescence

Studies rarely differentiate results based on viable and culturable bioaerosols, though only a proportion of microbial cells are culturable (Duquenne, 2018; Park et al., 2015). Culturable bioaerosols have been correlated to adverse health outcomes, including allergies, asthma, and other respiratory illness (Douwes et al., 2003; Kumar et al., 2013; Ross et al., 2000). However, the viable but not culturable (VBNC) proportion of bioaerosols can still pose health risks (Mainelis, 2019; Pearson et al., 2015; Speight et al., 1997). The viability state of bioaerosols can be monitored with a marker that detects the Adenosine triphosphate (ATP) contents in samples (Bajerski et al., 2018), and the analysis procedure is described elsewhere (T. T Han et al., 2018; T. Han, Wren, DuBois, Therkorn, & Mainelis, 2015; Seshadri, Han, Krumins, Fennell, & Mainelis, 2009). In short, 100 μ L of the aliquoted samples were combined with an equal volume of BacTiter-Glo reagent (Pro-mega Crop., Madison, WI). The tube was vortexed for ~5 sec and incubated at room temperature for 1 min. The luminometer (model 20/20n, Turner Biosystems Inc., Sunnyvale, CA) was then used to measure the luminescence intensity of the resulting aliquot in relative luminescence units (RLU). All samples were analyzed in triplicate, and the airborne ATP concentration, C_{ATP} (RLU/m³), was determined as follows:

where RLU is luminescence intensity; V_a is the volume aliquoted for the analysis; V_s is the entire sample volume in mL; Q is the flowrate in Liter/min; t is the sampling time in min.

4.3.5.3 Flow Cytometry (Live/Injured/Dead status)

Flow cytometry analysis with fluorescent dual stains quantifies the physiological states of a cell population, i.e., percentage of live, dead, injured, and unstained. This procedure is also described in T. T. Han et al. (2018). The procedure involves stock solution preparation, sample preparation, and sample analysis.

- Stock solutions preparation: cFDA-AM (5-Carboxyfluorescein Diacetate, Acetoxymethyl Ester; Life Technologies, Eugene, Oregon, USA) stock was prepared by dissolving 1 mg of cFDA- AM powder in 1 mL DMSO (Dimethyl sulfoxide; Life Technologies, Eugene, Oregon, USA) solvent and stored at -20 °C in the dark. The resulting stock concentration was 1.9mM of cFDA- AM solution. PI (Propidium Iodide; Life Technologies, Eugene, Oregon, USA) of 1.0 mM stock solution was prepared in distilled water from the supplier's solution of 1 mg/mL and stored at 4 °C in the dark.
- Sample preparation: Stock solutions were thawed and briefly vortexed before analysis. Triplicate 0.3 mL aliquots of samples were transferred into 1.5 mL centrifuge tubes. A final concentration of 50 µM cFDA-AM was added, followed by vortexing the tubes briefly, and then the samples were incubated in the dark at 37 °C for 30 min. After

incubation, 25 μ M of PI was added into the tubes and vortexed again (Jepras et al., 1995; King, 2000). Live and dead cell populations of samples were identified using single stained controls. Two different sources were used to prepare the live and dead cell controls. The first source was pure-cultured lab-grown bacteria and fungi diluted to an approximate concentration of $10^5 - 10^6$ cells/mL, as per microscopy; the second source was a blend of samples from all personal samplers. Only cFDA-AM was added to the live cell controls, and the solution kept in the dark at 37 °C for 30 min. The dead cell controls were prepared by killing the cells in a steaming water bath set at 80 °C for 20 min prior to staining by PI only. All samples and controls were kept on ice and analyzed within an hour from preparation.

• Sample analysis: Samples were analyzed using the BD Accuri C6 Flow Cytometer (BD Life Sciences, San Jose, CA). Samples were gated using side scatter (SSC) threshold, which represents the cell density or granularity (Müller & Nebe-von-Caron, 2010) and forward scatter (FSC) area (A) vs. height (H) plot to prevent the counting of doublets. Both dyes had an excitation wavelength of 488nm. The emissions of cFDA-AM at a wavelength of 530 ± 30 nm were captured by FL1 (fluorescence 530 nm bandpass filter), and the emissions of PI at a wavelength > 660 nm were captured by FL3 (fluorescence 660 nm bandpass filter) (Banin et al., 2006; Van Nevel, 2014). A plot was selected with filters FL1 vs. FL3. An unstained sample, live, and dead controls were evaluated initially to gate the positions of the unstained, live, and dead cells in the plot, respectively, followed by the samples. The proportions (%) of live, injured, dead, and unstained were determined from the plot. The two sources chosen for the controls verified the locations of live and dead cells on the plot.

Trypticase soy agar (Difco, Becton, Dickinson and Co., Sparks, MD) with fungicide cycloheximide (50 μ g/mL; Fisher Scientific Company Ltd., Hanover Park, IL) and malt extract agar (Difco, Becton, Dickinson and Co.,) were used as growth media for bacteria and fungi, respectively. 100 μ L of the 1 mL aliquot sample was pipetted to the center of a Petri-plate and spread out evenly using spreaders (Fisher Scientific Inc.). The plates were placed in an incubator for 72 hours at room temperature, and the colony forming units (CFU) were counted every 24 hours. Field and media blanks were also included for each sampling day, and any CFU counts on the blanks were subtracted from sampling data. Each sample was analyzed in triplicate, and the resulting airborne culturable concentration was calculated using the formula:

$$C_{culture} (CFU/m^3) = \frac{\frac{(\text{sum of the CFUs counted over the 72-hr period)} \times V_s \times 1000}{V_a} \dots (Eq. 3)$$

where CFU is the number colonies forming units counted on the Petri-plates; V_a is the volume aliquoted for the analysis; V_s is the entire sample volume in mL; Q is the flowrate in Liter/min; t is the sampling time in min.

4.3.6 Statistical Analysis

Since the majority of the data had a non-normal distribution, the data was log-transformed except flow cytometry results that were normally distributed. A full factorial analysis using a general linear model (GLM) was performed on the log-transformed or non-transformed variables to compare the main and interaction effects of the three investigated variables, i.e., site (n=3), sampler (n=8), and mannequin (n=2). Initial analysis showed that the two

units of each sampler placed on M1 and M2 performed identically to each other for the three sites (p > 0.05). Hence, the interaction effect of mannequins was omitted from the factorial analysis, and a two-way ANOVA was performed. Post-hoc test using Tukey-B was used to compare differences among the individual samplers. The average and standard deviation (SD) of the data are presented in tables and figures for each sampler stratified by the three sites. The bioaerosol concentrations stratified by the sites and samplers independently are in the format: mean \pm SD in the Results section. Statistical analysis was performed using IBM SPSS Statistics 26.0 (IBM, Armonk, NY), and statistical significance was accepted at p values < 0.05. OriginPro 2018 (OriginLab, Northampton, MA) was the graphing tool used to illustrate the data for this study.

4.4 Results and Discussion

4.4.1 Effect of the Site on Personal Exposures to Bioaerosols

One of the goals of the study was to investigate how different sites affect personal exposures to bioaerosols. Since the performance of individual personal samplers differed substantially, as explained below, the effect of site on personal exposures is best observed when the exposure data collected by the five personal samplers are pooled and stratified only by the sampling sites. The results in this Section 3.1 are presented for different exposure metrics: total bioaerosol concentration, ATP concentration, fractions of live and dead cells, and culturable bioaerosol concentrations. The same exposure metrics measured by individual personal samplers are analyzed in the subsequent Section 3.2.

4.4.1.1 Environmental Parameters at the Three Sites

The temperature and RH levels at the three sampling sites, i.e., horse barn, outdoor, and greenhouse, ranged from 3 $^{\circ}C - 22 ^{\circ}C$ and 35 % - 75%, respectively. The lowest

temperature and RH were measured outdoors, while the highest was measured in the greenhouse. The temperature inside the horse barn readings was 6 °C higher than the temperature outdoors, but they had similar relative humidity readings.

4.4.1.2 Total Number Concentrations of Bacteria and Fungi (#/m³)

The measured total number concentrations of bacteria (#/m³) varied significantly between the three sites (F = 57.727, p < 0.001) and are presented in Figure 4-3a, and Table C-1. Overall, the horse barn had the highest number concentrations, with a mean of 3.35 x 10⁶ #/m³ (\pm 2.04 x 10⁶), and they were significantly higher than the concentrations in the other two sites (p < 0.05): about 3x compared to the outdoor (1.36 \pm 0.96 x 10⁶ #/m³) and the greenhouse (1.09 \pm 0.62 x 10⁶ #/m³). The latter two sites had similar number concentrations (p > 0.05). For comparison, Prussin, Garcia, & Marr (2015) reported bacteria-like particle concentrations of 8.4 x 10⁵ #/m³ in an outdoor urban location, which was in the lower range compared to our outdoor site. In contrast, a dust plume over Beijing was observed to have bacterial concentrations up to 10⁸ #/m³ (Yuan et al., 2017), which was two magnitudes higher than the levels in our measurement locations.

Figure 4-3b and Table C-1 presents the measured total fungi number concentrations stratified by the three sites and the five samplers. The fungi number concentrations (#/m³) varied significantly between the sites as well (F = 39.289, p < 0.001). The outdoor site had the highest number concentrations ($8.95 \pm 6.21 \times 10^4$ #/m³; p < 0.05) and they were 2x higher than number concentrations in the greenhouse ($4.17 \pm 3.18 \times 10^4$ #/m³), whereas the horse barn had slightly lower fungi levels than the outdoor site ($7.08 \pm 4.14 \times 10^4$ #/m³; p < 0.05). Lee et al. (2006) reported outdoor fungi levels of up to 7.7 x 10³ spores/m³ using

the Button Sampler in the Midwestern United States (US); this result was one order magnitude lower than our outdoor site in the Northeastern US.

4.4.1.3 ATP Concentrations

The measured ATP concentrations (RLU/m³) stratified by the three sites, and the five samplers are presented in Figure 4-4 and Table C-2. The ATP concentrations varied significantly between the sites (F = 113.3, p < 0.001). The horse barn had the highest ATP concentrations ($4.68 \pm 4.17 \times 10^5 \text{ RLU/m}^3$), and it was 2x and 5x times higher than those in the greenhouse ($2.86 \pm 1.91 \times 10^5 \text{ RLU/m}^3$) and outdoors ($7.65 \pm 4.38 \times 10^4 \text{ RLU/m}^3$), respectively. The outdoor site had the lowest ATP concentrations, and that could be explained by environmental factors. It has been shown that lower temperature results in lower levels of viable bioaerosols (Lee et al., 2006; Shelton et al., 2002) and that results in lower ATP concentrations.

4.4.1.4 Proportions of Live, Injured, and Dead Cells

The proportions of live and dead cells in the collected samples stratified by the three sites and the five samplers are presented in Figure 4-5a and Figure 4-5b and their descriptive statistics are provided in Tables C-3 and C-4, respectively.

The proportion of the live cells varied significantly between the three sites (F = 10.742, p < 0.001), with higher percentages measured in the horse barn ($26.2 \pm 8.0 \%$) and the greenhouse ($24.8 \pm 10.7 \%$). Whereas, the outdoor site had fractions of live cells 6% less on the absolute scale than the other two sites, with a mean of 20.2 % (\pm 5.5). This result is consistent with the ATP concentrations described above.

The proportion of injured cells varied significantly between the sites as well (F = 33.946, p < 0.001). The outdoor site had the highest percentage of injured cells (52.9 ± 6.5 %). The horse barn and greenhouse had lower proportions of injured cells (p < 0.05), with a mean of 45.8 % (± 10.1) and 36.2 % (± 5.0), respectively. There is a higher probability for cells to become injured in natural open and semi-open environments due to oxidative stresses and UV radiations compared to a controlled closed environment, such as the greenhouse (Brągoszewska & Pastuszka, 2018; Lodovici & Bigagli, 2011).

In contrast to the live and injured cell results, the proportion of dead cells did not differ significantly among the sites (F = 2.458, p = 0.094). However, the outdoor site had the highest proportion of dead cells (16.1 \pm 6.03 %), followed by the greenhouse (15.5 \pm 5.8 %). The lowest fraction of dead cells was measured in the horse barn, with a mean of 13.5 % (\pm 6.8).

4.4.1.5 Culturable Concentrations of Bacteria and Fungi (CFU/m³)

The total culturable concentrations of bacteria and fungi stratified by the three sites and the five samplers are presented in Figure 4-6a and Figure 4-6b, respectively and their descriptive statistics are provided in Table C-5.

The three sites had significantly different total culturable concentrations of bacteria (F = 98.716, p < 0.001) and fungi (F = 27.923, p < 0.001). The measured culturable concentrations of bacteria and fungi in the horse barn (culturable bacteria: 4818 ± 4721 CFU/m³; culturable fungi: 447 ± 227 CFU/m³) were 20x and 3x higher than those measured outdoors (culturable bacteria: 240 ± 295 CFU/m³; culturable fungi: 126 ± 157 CFU/m³). The horse barn also had higher culturable bacteria (~60x) and fungi (~1.5x) levels than those of the greenhouse (culturable bacteria: 72 ± 67 CFU/m³; culturable fungi: 282 ± 203

 CFU/m^3). The measured culturable bacteria concentrations in the greenhouse were 3x lower than in the outdoor site, despite having a comparable total bacteria concentration.

Samadi et al. (2009, 2012) reported culturable bacteria and fungi concentrations from 10³ to 10⁴ CFU/m³ and 10² to 10³ CFU/m³ in horse stables, respectively; these concentrations were similar to the horse barn site in our study. Mbareche, Veillette, Bilodeau, & Duchaine (2019) measured culturable fungi concentrations of 10⁶ CFU/m³ during summer in a dairy farm, which was significantly higher than in our horse barn site.

Other studies of the ambient environment have reported comparable culturable concentrations to our outdoor site. A study by Naddafi et al. (2011) measured an average bacterial level of 268 CFU/m³ in the outdoor air of Tehran near to metro stations. Zhu et al. (2003) reported culturable bacteria concentrations in the ambient air ranging from 200 – 850 CFU/m³ in Southern US and Crawford et al. (2015) reported culturable fungi concentration in the range of 10 - 800 CFU/m³ in Northeastern US during the fall season; the measured culturable bioaerosol concentrations at the outdoor site in our study were within these ranges. However, a study of 2407 outdoor samples from different locations across the US had a median culturable fungi concentration of almost 4x higher than the average value of our outdoor site (reported study vs. our study: 540 CFU/m³ vs. 126 CFU/m³) (Shelton et al., 2002).

4.4.1.6 Summary of Exposure Data at the Three Sampling Sites

The horse barn representing a semi-open animal farm site had the highest bacteria number concentrations, culturable bioaerosol concentrations, ATP concentrations, as well as fractions of live cells. In contrast, the outdoors had the lowest ATP concentrations, fractions of live cells, and culturable fungi concentrations. The highest fractions of injured and dead cells were also measured outdoors. We conclude that the environmental conditions and bioaerosol sources at the three sites—forming unique microenvironments—had a significant effect on personal bioaerosol exposure (Lighthart, 2000; Zhu et al., 2003).

4.4.2 Biological Performance of the Personal Samplers

The five selected personal samplers differ in their flow rates (1 - 10 L/min), the collection mechanisms (centrifugal impaction, electrostatics, and filtration), collection media (dry tubes, filter, and liquid), and sample elution process. Since the performance of individual samplers varied site-to-site (Figures. 4-3 to 4-6), the sampler effect on the personal bioaerosol exposures measured using different metrics was analyzed using data pooled across the three sites and stratified by the samplers.

4.4.2.1 Total Number Concentrations of Bacteria and Fungi (#/m³)

The five personal samplers measured significantly different total number concentrations (#/m³) of bacteria (F = 34.702, p < 0.001; Figure 4-3a) and fungi (F = 69.011, p < 0.001; Figure 4-3b). NIOSH-T sampler measured the highest number concentrations of bacteria and fungi, with a mean of $3.26 \pm 2.30 \times 10^6$ #/m³ and $1.21 \pm 0.44 \times 10^5$ #/m³, respectively. Measurements with UPAS yielded number concentrations similar to those measured by NIOSH-T sampler: the mean concentration of bacteria was $2.28 \pm 1.31 \times 10^6$ #/m³, and the mean concentration of fungi was $1.08 \pm 0.45 \times 10^5$ #/m³. This result is unexpected because UPAS has a cut-off size of 2.5 µm, and NIOSH-T sampler has an aspiration efficiency of 96% for particles of 6.2 µm and \geq 98% for particles up to 3.1 µm (Lindsley et al., 2006). However, the Prospective Urban Rural Epidemiology (PURE) study reported the first version of UPAS (v1.0) to have overestimated the PM_{2.5} concentrations compared to the Harvard impactor (Air Diagnostics & Engineering Inc., Harrison, ME) (Arku et al., 2018).

More testing of UPAS will be needed to provide insights into its performance as a bioaerosol sampler, as neither its previous (v1.0) not its current (v2.2) version has been tested for bioaerosol sampling prior to this study.

On the other hand, the lowest number concentrations were measured by CIP 10-M sampler (mean concentration of bacteria: $8.43 \pm 9.19 \times 10^5 \text{ #/m}^3$; mean concentration of fungi: $2.39 \pm 1.14 \times 10^4 \text{ #/m}^3$). These concentrations were up to ~5x lower than those determined by the NIOSH-T and UPAS samplers. This result is different from the one reported by Görner et al. (2006), where the CIP 10-M sampler reported total microbial cell concentrations of $10^6 - 10^7$ cells/m³ in a waste treatment plant and that was similar to results produced by a 37 mm filter cassette (Millipore, Darmstadt, Germany).

The Button sampler measured total bioaerosol concentrations ~2x lower than that those measured by NIOSH-T and UPAS samplers, with mean concentrations of 2.13 ± 1.53 x 10^6 #/m^3 and $5.56 \pm 2.53 \times 10^4 \text{ #/m}^3$ for bacteria and fungi, respectively. This result is different from a study that reported a comparable performance between the sum of the three stages of the NIOSH sampler and a personal inhalable sampler (PGP sampler, DEHA Haan & Wittmer, Heimsheim, Germany) for total concentrations of bacterial cells in a poultry production facility (Martin et al., 2015). A study by Blais Lecours et al. (2012) also reported no statistical difference between the sum of samples in the three stages of the NIOSH sampler and the samples eluted from a personal inhalable IOM sampler (SKC Inc.) for the results of the 16s rRNA gene concentrations measured in a dairy barn.

PEBS measured bioaerosol concentrations of up to ~1.5x higher than those measured by CIP 10-M sampler, with mean values of $1.20 \pm 0.91 \times 10^6 \text{ #/m}^3$ and $2.95 \pm 1.23 \times 10^4 \text{ #/m}^3$ for total bacteria and fungi concentrations, respectively. T. T. Han et al.

(2018) reported a similar result where PEBS measured higher airborne microbial concentrations compared to a liquid-based sampler (BioSampler, SKC Inc.) for both short (10 mins) and long term (4 hours) sampling. However, the total bioaerosol concentrations measured by PEBS were lower than those measured by NIOSH-T, UPAS, and Button samplers (p < 0.05). This is most likely due to lower physical collection efficiency of PEBS compared to filter samplers (T. T. Han et al., 2017).

Due to limited literature on the performance of personal bioaerosol samplers and in order to make meaningful comparisons, we drew parallels between the CIP 10-M sampler and BioSampler (SKC Inc.), another liquid bioaerosol sampler. Both samplers collect particles directly into liquid medium: into a rotating cup for the CIP 10-M and swirling liquid for the BioSampler. These two samplers also have similar collection efficiencies for particles of sizes similar to those of typical bacterial and fungal spores: the CIP 10-M sampler with the inhalable head follows the inhalable convention curve recommended by the American Conference of Governmental Industrial Hygienists (ACGIH) (Görner et al., 2006), which means it has aspiration efficiency close to 100% for particles $< 5 \,\mu$ m, whereas the BioSampler has a collection efficiency close to 100% for > 1.5 μ m (Daher et al., 2011) and has been previously used to determine exposures to inhalable particles (Haas et al., 2010; Hogan et al., 2005). Additionally, Cassier et al. (2013) studied the inhalation exposure of Legionella bacteria aerosolized from hospital washbasin water using both the CIP 10-M sampler and BioSampler (SKC Inc.). Legionella spp. and Legionella *pneumophila* were detected in samples retrieved from both the liquid-based samplers.

4.4.2.2 ATP Concentrations Reported by the Personal Samplers

Similar to the total number concentrations of bacteria and fungi, the airborne ATP concentrations (ATP/m³) of the measured bioaerosols varied significantly between the samplers (F = 21.35, p < 0.001; Figure 4-4). The ATP concentrations measured by CIP 10-M, UPAS, and NIOSH samplers have not been reported in other studies, and, therefore, comparison with other studies when using this metric is not available.

The highest ATP concentrations were measured by the NIOSH-T sampler (6.30 \pm 5.17 x 10⁵ RLU/m³). The other samplers measured significantly lower ATP concentrations compared to NIOSH-T sampler, yet on par with each other: CIP 10-M sampler yielded the mean concentration of 2.14 \pm 1.42 x 10⁵ RLU/m³, PEBS yielded mean concentration of 1.94 \pm 1.17 x 10⁵ RLU/m³, Button sampler measured a mean concentration of 1.83 \pm 1.39 x 10⁵ RLU/m³, and UPAS produced a mean concentration of 1.63 \pm 1.15 x 10⁵ RLU/m³ (p > 0.05).

The literature on ATP concentrations measured by personal samplers is very limited. The existing bioaerosol studies that measured ATP indicate that the yielded ATP concentration depends on the sampling method (T. Han et al., 2015). However, due to such limitations, we again draw parallels between the liquid-based samplers, i.e., CIP 10-M sampler and BioSampler (SKC Inc.), as mentioned in Section 3.2.1. The similar ATP concentrations measured by CIP 10-M, PEBS, and Button samplers in our field investigations is a different result from the previous laboratory studies where higher ATP concentrations of pure-cultured lab samples of *B. atrophaeus* and *P. fluorescens* were measured by the Button sampler compared to the liquid-based BioSampler (SKC Inc.) (T. Han et al., 2015). Another study also reported that PEBS measured significantly higher

ATP concentrations of *B. atrophaeus bacteria* but lower ATP concentrations of fungus *P. chrysogenum* compared to BioSampler (SKC Inc.) (T. T. Han et al., 2018). However, it is important to stress that these studies measured laboratory-generated organisms, and our study looked at bioaerosols in the field, where they might be hardened by environmental stressors and less sensitive to variability due to sampling stress produced by different samplers.

4.4.2.3 Proportions of Live, Injured, and Dead Cells

The live, injured, and dead cell fractions of the bioaerosols eluted from the three stages of the NIOSH sampler (NIOSH-L, NIOSH-S, and NIOSH-F stages) are reported separately since all analyzes of the individual stages were performed separately, and fractions from the three stages do lend themselves to be combined into a single parameter.

The proportion of live cells differed significantly between the samplers (F = 18.693, p < 0.001; Figure 4-5a). The highest fraction of live cells was recovered by PEBS, with a mean of 37.5 ± 8.4%). Previously, PEBS was reported to have measured similar live cells fraction of *B. atrophaeus* and *P. chrysogenum* to that of a liquid-based sampler (BioSampler, SKC Inc.) for both short and long term sampling (T. T. Han et al., 2018). This study showed that PEBS recovered significantly higher live cell fractions than the other samplers (p < 0.05), while the remaining four personal samplers performed on par with each other (p > 0.05). Among the personal samplers other than PEBS, the highest fraction of live cells was measured by the NIOSH-L stage (23.6 ± 4.9 %) and UPAS (22.8 ± 4.6 %). The average values of live cells fractions measured by Button sampler (22.6 ± 8.0 %), NIOSH-F stage (20.1 ± 6.1 %), and CIP 10-M sampler (19.9 ± 7.5 %) were marginally higher than that of the NIOSH-S stage (19.4 ± 5.7 %).

The five personal samplers yielded similar fractions of injured cells (F = 1.072, p = 0.389), even though they had different collection mechanisms and media. When the values of the injured cell fractions results were pooled together, the samplers had an overall mean of $45.0 \pm 10.1\%$.

The proportions of dead cells varied significantly between the samplers as well (F = 8.830, p < 0.001; Figure 4-5b). The lowest fraction of dead cells was measured by PEBS ($6.4 \pm 3.8 \%$), and this result was significantly different from that of the other samplers (p < 0.05). Similar to the fractions of live cells, the remaining four personal samplers performed similarly to each other (p > 0.05). NIOSH-L stage retrieved the second-lowest dead cells fraction ($14.9 \pm 3.4 \%$), whereas the NIOSH-S stage ($18.5 \pm 4.0 \%$) and CIP 10-M sampler ($17.6 \pm 7.1 \%$) retrieved higher fractions of dead cells. Damage due to the impaction and desiccation of bioaerosols, when collected on filters, explains the relatively higher fractions of dead cells for NIOSH-F stage ($16.8 \pm 6.6 \%$), Button sampler ($15.5 \pm 6.2 \%$) and UPAS ($15.2 \pm 4.2 \%$) (Lindsley et al., 2017; Zhen et al., 2013).

All three stages of the NIOSH sampler, as well UPAS, CIP 10-M, and Button samplers had unstained cell fractions similar to each other $(16 \pm 8 \%)$, except for PEBS, which had with the lowest unstained cell fraction (p < 0.05; 8 ± 4%) (data not shown).

4.4.2.4 Culturable Concentrations of Bacteria and Fungi (CFU/m³)

The five personal samplers measured significantly different concentrations of culturable (CFU/m^3) of bacteria (F = 2.941, p = 0.031; Figure 4-6a). The CIP 10-M sampler measured the lowest culturable concentrations, with a mean of 641 CFU/m³ (± 1152), and it was significantly lower than the concentration measured by the NIOSH-T sampler (4194 ± 6406 CFU/m³; p < 0.05). Görner et al. (2006) reported concentrations of culturable bacteria

100x lower than the total microbial concentrations in an urban waste-sorting industry; however, our findings indicate ~1000x lower culturable bacteria concentrations compared to total the bacterial concentrations when using the CIP 10-M sampler. The lower culturability of samples collected by the CIP 10-M sampler could be attributed to mechanical stress experienced by the biological particles upon impingement and exacerbated by desiccation once the collection liquid level is reduced due to evaporation during sampling (Duquenne et al., 2012; Görner et al., 2006; Mainelis, 2019; Simon et al., 2016).

The remaining four samplers performed not significantly different from each other in terms of measured culturable bacteria concentrations (p > 0.05), even though the average concentrations yielded by PEBS (1174 ± 1703 CFU/m³) and Button sampler (2229 ± 2976 CFU/m³) were 5 – 10x higher than those measured by UPAS (312 ± 448 CFU/m³). These culturable bacteria concentrations were comparable to the results produced by the NIOSH-T sampler, even though the PEBS and Button samplers measured significantly lower number concentrations of bacteria compared to the NIOSH-T sampler. This indicates higher culturability of samples collected by the PEBS and Button samplers.

The five personal samplers measured significantly different total culturable fungi concentrations as well (F = 5.636, p < 0.001; Figure 4-6b). NIOSH-T sampler measured the highest culturable concentrations of fungi (389 ± 229 CFU/m³), while CIP 10-M sampler (196 ± 190 CFU/m³) and PEBS (142 ± 155 CFU/m³) measured the lowest culturable concentrations of fungi (p < 0.05). UPAS and Button samplers had mean concentrations of 318 ± 200 CFU/m³, and 379 ± 299 CFU/m³, respectively, and they were comparable to the culturable fungi concentrations measured by NIOSH-T sampler (p >

0.05). Lower culturable fungi concentrations measured by PEBS are likely due to the locally strong electrostatic fields formed on the irregular surfaces of fungal spores, making the spores less culturable (T. T. Han et al., 2018). Overall, the performance of five personal samplers in terms of the culturable fungi concentrations followed a similar trend to the total fungi number concentrations, which indicates that the culturable fractions of fungi measured by all samplers were not different.

4.4.2.5 Summary of the Biological Performance of the Five Personal Samplers

In general, the highest total fungi and bacteria concentrations were measured by the NIOSH-T sampler. However, samples recovered from PEBS had the highest fraction of live cells and the lowest fraction of dead cells. The culturable cell concentrations measured by electrostatic sampler PEBS were also comparable to those measured by other personal samplers. The CIP 10-M sampler yielded the lowest total and culturable bioaerosol concentrations. Furthermore, our findings suggest that, on average, the total bioaerosols concentrations measured by UPAS were not different from those measured by the other samplers.

The potential advantages and disadvantages of each personal sampler during field use, as perceived by the researchers in this study are described in Table 4-2. We hope these insights will be useful in future studies when selecting a personal sampler for specific sampling environments and target microorganisms. Our study also adds to the currently limited literature on personal sampler performance and comparisons when measuring exposures to bioaerosols.

4.4.3 Comparison of the Three Stages of NIOSH Sampler

The NIOSH cyclone-based sampler is capable of providing information on different bioaerosol size fractions (Lindsley et al., 2017). We used this sampler's capability to compare bioaerosol fractions in our study as well, and the data are presented in Table 4-3. The fractions for each metric was calculated as the yield of each stage relative to the sum of yields from the three stages, except for viable and dead fractions that were calculated separately for each stage. The first stage of the NIOSH sampler (NIOSH-L), which collected particles $> 4 \mu m$ in size in a dry centrifuge tube, measured the highest fractions of total bioaerosol concentrations (bacteria: $56 \pm 6\%$; fungi: $50 \pm 7\%$), ATP concentrations $(61 \pm 9 \%)$, and culturable concentrations (bacteria: $85 \pm 11 \%$; fungi: $53 \pm 7 \%$). The highest fractions of live cells (24 ± 5 %) and the lowest fractions of dead cells (15 ± 3 %) were also recovered from the NIOSH-L stage. These findings are similar to the study by Lighthart (1997) and Martin et al. (2015) that suggested that most airborne microbes are present as particles larger than 3 μ m and 5 μ m, respectively. In addition, aggregation of biological cells and their attachment to larger-sized particles (Martin et al., 2015) offer protection from solar radiation damage in the ambient air (Fröhlich-Nowoisky et al., 2016). Our observations are consistent with the latter two studies.

The second stage of the NIOSH sampler (NIOSH-S), which collected particles from $1-4 \mu m$ in size, had slightly lower fractions of bacterial number concentrations ($40 \pm 2\%$) compared to those yielded by the NIOSH-L stage ($56 \pm 6\%$; p > 0.05), yet the fractions of culturable bacteria of NIOSH-S ($15 \pm 11\%$) were significantly lower than that of NIOSH-L stage ($85 \pm 11\%$; p < 0.05). The fractions of fungi number concentrations ($30 \pm 7\%$) and their culturability ($31\pm 7\%$) were also lower in stage NIOSH-S than those of the NIOSH-

L stage. Additionally, the proportion of ATP concentrations $(34 \pm 9 \%)$ was ~50% lower than those in the NIOSH-L stage $(61\pm 9 \%)$. The proportions of viable and dead cells at the NIOSH-S stage (live cells: $19 \pm 6 \%$; dead cells: $19 \pm 3 \%$) were similar to those in the NIOSH-F stage (live cells: $20 \pm 7 \%$; dead cells: $17 \pm 4 \%$) and lower than in the NIOSH-L stage (live cells: $24 \pm 5 \%$; dead cells: $15 \pm 3 \%$). These results indicate that the viability of smaller-sized bioaerosols, which could be single cells or small aggregates, in ambient environments is reduced compared to larger bioaerosols. Reduction in viability could be due to the dehydration of individual cells upon aerosolization (Wyatt & Phillips, 1972), damage by solar radiation, and low moisture content (Fernandez et al., 2019). Also, as indicated above, larger aggregates offer more protection from solar radiation.

The third stage of the NIOSH sampler (NIOSH-F), which collected the smallest particles, i.e., < 1 μ m in size, measured the lowest proportions of bioaerosol number concentrations (bacteria: 8 ± 2%; fungi: 20 ± 7%) compared to the other two sampler stages. Lindsley et al. (2006) reported that less than 0.4% of *Aspergillus* and *Penicillium* fungi spores was measured by the NIOSH-F stage compared to the total fungal count. Our findings indicated that 20% of the total fungi concentration collected by the NIOSH sampler was eluted from its third stage. However, the lowest fraction of ATP concentrations was recovered from the NIOSH-F stage (5 ± 3 %). The fractions of culturable bioaerosol concentrations (bacteria: 0.2 ± 0.3 %; fungi: 17 ± 3 %) were significantly lower than those from the NIOSH-L stage (p < 0.05) and similar to the NIOSH-S stage (p > 0.05). These results indicate that the exposure to particles bacterial and fungal particles were generally lower compared to the exposure to particles > 1 μ m. The viability and culturability of single-organism bioaerosols or smaller

aggregates could have been further reduced by the damage due to desiccation once collected on filters (Durand et al., 2002; C.-H. Wang et al., 2015).

4.5 Conclusions

Personal bioaerosol exposures differed at the three sites in terms of measured total bacteria and fungi number concentrations, ATP concentrations, including fractions of live and dead cells, and culturable concentrations. The environmental conditions and bioaerosol sources at the three sites had a significant effect on personal bioaerosol exposures. Intra-variability in terms of biological performance between the two sets of each personal sampler was not statistically significant (p > 0.05). The collection mechanisms, collection media, and sample elution process of the five personal samplers likely influenced their ability to measure personal bioaerosol exposures in the investigated environments. We found that the dry cyclonic tubes (NIOSH sampler) and filter-based samplers (i.e., Button and UPAS samplers) measured the highest total bioaerosol concentration. Samples eluted from PEBS had the highest percentage of live cells and comparable culturable bioaerosol concentrations to the other four samplers. UPAS, which is $PM_{2.5}$ sampler, yielded total and culturable bioaerosol concentrations similar to that of other samplers. Additionally, the NIOSH sampler provided information on three bioaerosol size fractions. These findings, along with the field-acquired experience of the advantages and limitations of the five personal samplers, could facilitate future studies into the development and selection of personal bioaerosol samplers for various studies. Additional studies will explore the speciation of bioaerosols recovered by these personal samplers and measuring personal bioaerosol exposures in occupational and residential locations.

4.6 Acknowledgments

CDC/NIOSH grants R21OH10560 and R01OH009783. The funding sponsor had no role in the design of the study, the collection, analysis, or interpretation of data, in the writing of the manuscript, and in the decision to publish these results. The authors would like to sincerely thank the Rutgers Horse Barn Facility and the Rutgers Greenhouse for facilitating access to their locations and the Rutgers SEBS Core instrumentational facility for access to the C6 Flow Cytometer. We would also like to acknowledge Dr. William G. Lindsley (CDC/NIOSH) for providing the NIOSH BC 251samplers.

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Figure 4-1. Field study sites selected to measure personal bioaerosol exposure using five personal samplers attached on two mannequins a) Site 1: Horse Barn b) Site 2: Outdoors c) Site 3: Greenhouse

Sampler Name	Flow	Captured	Mechanism and
	rate	Particle	medium used for
		Fraction	particle collection
Field-deployable Personal Electrostatic	10 L/min	No size selective inlet	Electrostatic precipitation
Bioaerosol Sampler (PEBS; in-house			collects particles on a
sampler)			superhydrophobic surface
CIP 10-M sampler (CIP 10-M, Air Sampling	~10 L//min	Inhalable fraction	Cyclonic collection into
Devices, Milford, NH) with built-in pump	(7000 rpm)		liquid (PBS in this study)
- Designed for bioaerosol sampling			
Ultrasonic Personal Aerosol Sampler (UPAS,	1 L/min	PM _{2.5} fraction	Filter-based sampling onto
Access Sensor Technologies, Fort Collins,			37 mm filter (PTFE with 2
CO) with built-in pump			µm pore size, SKC, Eighty
			Four, PA used in this
- Not specifically designed for bioderosol			study).
sampling			
NIOSH Personal Biogerosol Cyclone	351/min	a < 1 um (NIOSH)	Two dry cyclones and a
	5.5 L/IIIII	$\begin{bmatrix} a. & \langle 1 \ \mu m (NOS) \\ F \end{pmatrix}$	
Sampler 251 (NIOSH, Morgantown, VA)		b. 4 to 1 µm (small	37mm filter (PTFE with a
with external pump		tube) (<i>NIOSH-S</i>)	2 μm pore size, SKC Inc.
		c. > 4 μ m (large tube) – (<i>NIOSH</i> - <i>L</i>)	used in this study)
		d. $NIOSHT =$	
		NIOSH-F + NIOSH-S + NIOSH-L	
Button Aerosol Sampler (SKC, Eighty Four,	4 L/min	Inhalable fraction	25mm filter (PTFE with
PA) with external pump			0.45 µm pore size, SKC
			Inc.)

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Figure 4-2. A prototype of the personal bioaerosol electrostatic sampler (PEBS), the power

controller, and the field test setup using a mannequin.



b)



Figure 4-3. a) total bacteria number concentrations (#/m³) for five personal samplers measured by the acridine orange staining method using epifluorescence microscopy b) total fungi number concentrations (#/m³) for five personal samplers measured by hemocytometer chamber viewed under a direct microscope. The data shown were primarily stratified by the three sites (horse barn, outdoors, and greenhouse) followed by the samplers (Button, PEBS, CIP 10-M, UPAS, NIOSH-L, NIOSH-S, NIOSH- F, and NIOSH-T). The data points are an average of four repeats sampled at each site for two consecutive days, and the error bar represents standard deviation. Each repeat was analyzed in triplicates.


Figure 4-4. Adenosine triphosphate (ATP) concentrations in Relative Light Units (RLU/m³) measured by five personal samplers. The data shown in the log scale were primarily stratified by the three sites (horse barn, outdoors, and greenhouse) followed by the samplers (Button, PEBS, CIP 10-M, UPAS, NIOSH-L, NIOSH-S, NIOSH- F, and NIOSH-T). The data points are an average of four repeats sampled at each site for two consecutive days, and the error bar represents standard deviation. Each repeat was analyzed in triplicates.







Figure 4-5. a) proportion of live cells (%) and b) proportion of dead cells (%) analyzed by flow cytometry for samples retrieved from five personal samplers. The data shown in linear scale were primarily stratified by the three sites (horse barn, outdoors, and greenhouse) followed by the samplers (Button, PEBS, CIP 10-M, UPAS, NIOSH-L, NIOSH-S, and NIOSH-F). The data points are an average of four repeats sampled at each site for two consecutive days, and the error bar represents standard deviation. Each repeat was analyzed in triplicates.





b)

a)

Figure 4-6. a) culturable bacteria cell concentrations (CFU/m³) and b) culturable fungi cell concentrations (CFU/m³) measured by five personal samplers. The data shown in the log scale were primarily stratified by the three sites (horse barn, outdoors, and greenhouse) followed by the samplers (Button, PEBS, CIP 10-M, UPAS, NIOSH-L, NIOSH-S, NIOSH-F, and NIOSH-T). The data points are an average of four repeats sampled at each site for two consecutive days, and the error bar represents standard deviation. Each repeat was analyzed in triplicates. No colonies grew for NIOSH-F at outdoor and greenhouse locations for culturable bacteria concentrations.

Sampler	Advantages	Limitations
PEBS CIP 10-M	 Easy to use Limited on-site work Quick sample retrieval steps Quiet operation High saturation limits High flow rate sampler Samples collected directly into the preferred collection liquid Minimum steps needed for sample retrieval 	 Single unit portable version is in progress Fungi removal was not optimized – sample retrieval protocol has been revised for later studies Liquid needs to be added on- site to compensate for evaporation Higher risk of contamination
UPAS	 No sample saturation limit Easy to use Wireless connectivity to start the sampler Built-in timer Quiet operation Intermittent or continuous sampling 30+ hr battery life GPS enabled 	 Additional steps and longer time required to elute samples from the filters Only PM_{2.5} inlet currently available Currently advertised only as an indoor sampler The provided app does not control the sampler once sampling is in progress
NIOSH	 Dry sampling No on-site work Dry tubes can be easily sealed Ability to collect three particle size fractions 	 Labor intensive setup prior to sampling Additional steps and longer time required for sample retrieval from filters and two dry tubes Needs external pump, which could be cumbersome Sampling lines are needed to connect sampler and pump, which could be inconvenient for personnel
Button	 Easy to handle Omnidirectional inlet Sampler often used in PM exposure studies 	 Additional steps and longer time required to elute samples from the filters Needs external pump, which could be cumbersome Sampling lines are needed to connect sampler and pump, which could be inconvenient for personnel

Table 4-2. User experience with the five investigated personal samplers during field use.

Table 4-3. Bioaerosol fractions (in percentages) measured by each of the three sections of the NIOSH personal bioaerosol cyclone sampler 251 relative to the sum of the three sections (NIOSH- T). 1st stage: NIOSH-L, 2nd stage: NIOSH-S, and after - filter: NIOSH-F. Cell viability in terms of live and dead cells were measured separately in each stage.

Metric	1 st stage	2 nd stage (NIOSH-	After - filter
	(NIOSH-	S)	(NIOSH-F)
	L)		
Bacteria number	$56 \pm 6\%$	$40 \pm 2\%$	$8\pm2\%$
concentration (#/m ³)			
Fungi number	$50\pm7\%$	$30\pm7\%$	$20\pm7\%$
concentration (#/m ³)			
ATP concentrations	61 ± 9 %	34 ± 9 %	5 ± 3 %
(RLU/m ³)			
Live cells	24 ± 5 %	19 ± 6 %	20 ± 7 %
Dead cells	15 ± 3 %	19 ± 3 %	17 ± 4 %
Culturable bacteria	85 ± 11 %	15 ± 11 %	0.2 ± 0.3 %
concentration (CFU/m ³)			
Culturable fungi	53 ± 7 %	31±7 %	17 ± 3 %
concentration (CFU/m ³)			

CHAPTER 5. SUMMARY, IMPLICATIONS, AND FUTURE DIRECTIONS

5.1 Summary

Assessment of exposure to aerosols and bioaerosols is among the diverse, challenging, and complex studies. The dissertation is focused on evaluating exposures to aerosols and bioaerosols in different environments and attempts to form cohesive multi-dimensional analyses from diverse data streams. The main aims were accomplished by using advanced measurement tools and interdisciplinary approaches, for both area and personal aerosol sampling. These research aims are presented as the three main chapters of this dissertation and are as follows: 1) field study investigating indoor air quality (IAQ) determinants using interview data, building deficiencies, and IAQ measurement techniques 2) bioaerosol variability in multi-apartment residential buildings as a function of building factors and environmental parameters affecting the bioaerosol presence, and 3) evaluating personal exposures to bioaerosols in different environments using five different personal samplers.

5.1.1 Investigation of Indoor Air Quality Determinants in a Field Study Using Three Different Data Streams (Chapter 2; Area Sampling of Aerosols)

The integration of terrestrial laser scanning and infrared thermography with traditional air sampling and questionnaire usage identified various housing-related health and IAQ issues (Thomas et al., 2019). Building deficiencies were detected from 1609 infrared images, and missing wall insulation was identified from these images as patches with well-defined edges (Balaras & Argiriou, 2002). Real-time IAQ monitors measured particle number and mass concentrations and environmental parameters. Residents were asked during individual interviews about their perception of building quality and comfort, household

activities that could impact indoor air quality, and health problems in their family, such as asthma events and other illnesses.

Higher concentrations of indoor ultrafine particles (< 300 nm) were measured in apartments missing more than 5% of wall insulation. The missing insulation (MI) created a temperature gradient between indoor and outdoor spaces leading to tangential airflow, thus creating multiple air entry zones. In addition to outdoor sources, particles generated by residents indoors contributed to the overall presence and accumulation of particles. When the apartments were stratified by the levels of MI and indoor combustion sources, ultrafine particles were significantly higher in apartments with more than 5% MI and no or relatively low presence of smoking and burning of candles or incense. Corner apartments had a higher fraction of MI compared to non-corner apartments. Additionally, higher levels of MI were detected in apartments where a resident had an asthma attack in the past 12 months.

In conclusion, the integration of interdisciplinary data streams provided a more comprehensive assessment of building structural conditions, and its relation to indoor environmental parameters and residents' well-being.

5.1.2 Presence and Variability of Culturable Bioaerosols in Three Multi-Apartment Residential Buildings with Different Ventilation Systems in the Northeastern US (Chapter 3; Area Sampling of Bioaerosols)

This chapter was aimed at reducing the knowledge gap of exposures to indoor bioaerosols in multi-apartment residential buildings since we spend 70% of our time in residences (de Kluizenaar et al., 2017). We investigated the presence and variability of indoor culturable bacteria and fungi in three multi-apartment buildings located in the Northeastern United States (US). A total of 409 indoor and 86 outdoor samples were collected in 48 unique apartments that had two kinds of ventilation system: "central heating, ventilation and air conditioning (HVAC)" that supplied 100% conditioned outdoor air and "window air conditioning (AC)" units with natural ventilation and hot water baseboard heating.

The measured culturable bioaerosols and their indoor/outdoor ratios were similar to previous studies reported in the US and other countries (Lee et al., 2006; Moon et al., 2014; Shelton et al., 2002). Eighty-five percent of the investigated apartments had culturable fungi indoor-outdoor (I/O) ratios below 1, and these ratios were lower for the "central HVAC" apartments compared to the "window AC" apartments. This relationship held for all four seasons. However, the indoor bacteria concentrations and their I/O ratios measured in the three buildings had similar concentrations of culturable bacteria, regardless of the type of ventilation system. Overall, the observed I/O ratios suggested minimal indoor sources of fungi and the prominence of indoor sources of bacteria.

The dew point (DP), an environmental parameter that reflects water content in the air (Grinn-Gofroń et al., 2011; Ryan et al., 2002), was better controlled in the "central HVAC" apartments with humidification in winter and dehumidification in summer and was within the comfort zone (45°F to 55°F). Whereas, the "window AC" apartments that do not have humidification in winter and dehumidification in summer were subjected to extreme DPs. Extreme DPs could be related to health problems, including skin irritation and upper respiratory symptoms (Reinikainen & Jaakkola, 2003). The indoor culturable bioaerosol concentrations pooled together from the three buildings had a significant positive association with indoor DPs, and the relationship was identical even after stratifying the apartments by their ventilation systems.

In summary, we believe that the reported bioaerosol concentrations could be used as baseline readings to determine indoor air quality standards or recommendations in residential apartment buildings, and the approach of including dew point and seasonal variability provided a comprehensive relationship between the buildings' ventilation systems and indoor bioaerosols.

5.1.3 Evaluation of Personal Exposures to Bioaerosols Using Five Different Personal Samplers in Several Environments (Chapter 4; Personal Sampling of Bioaerosols)

Exposure to bioaerosols has been associated with respiratory tract illness (Falkinham, 2003), cardiovascular 2018), infections diseases (Dabass et al., and in immunocompromised persons and the general population (Hansen et al., 2012). The ubiquitous presence of bioaerosols and their negative health effects make it important to monitor and characterize bioaerosols, including their size distribution, species, viability, and culturability status. Common practices for bioaerosol exposure assessments utilize stationary and portable samplers. However, these samplers can represent an individual's personal exposure inaccurately (Mainelis, 2019; Wang et al., 2015). Thus, we chose to investigate the performance of five personal samplers to determine personal bioaerosol exposure and compared their inter and intra-variabilities in different environments.

The samplers compared in this study were: Personal Electrostatic Bioaerosol Sampler (PEBS; Rutgers University), CIP 10-M sampler (CIP 10-M; Air Sampling Devices, Milford, NH), Ultrasonic Personal Aerosol Sampler (UPAS; Access Sensor Technologies, Fort Collins, CO), National Institute for Occupational Safety and Health Personal Bioaerosol Cyclone Sampler 251 (NIOSH; NIOSH, Morgantown, VA) and Button Aerosol Sampler (Button; SKC, Eighty Four, PA). A set of these samplers was placed each on two mannequins in their chest region to measure personal bioaerosol exposures. The three selected locations, i.e., such as the horse barn, an outdoor site and the greenhouse, had different environmental factors and bioaerosol sources.

Our results indicated that bioaerosol exposure varied significantly between the sites (p < 0.05), and intra-variability between the two sets of each sampler was not statistically significant (p > 0.05). The NIOSH, Button, and UPAS samplers measured higher bioaerosol number concentrations compared to the liquid-based sampler (CIP 10-M), whereas the samples eluted from PEBS had the highest percentage of live cells. The samplers, except CIP 10-M, measured similar culturable bacteria concentrations (p > 0.05). UPAS measured culturable bioaerosol concentrations lower than that of the NIOSH sampler, though with similar total number concentrations. Additionally, the NIOSH sampler provided information on three bioaerosol size fractions. We conclude that the choice of a personal bioaerosol sampler, as well as its interaction with the exposure measurement metric, would likely affect the determined bioaerosol exposures. Our findings, along with the field-acquired experience of the advantages and limitations of the five personal samplers, could facilitate future studies into the development and selection of personal bioaerosol samplers for various studies.

5.2 Practical Implications

The method of integrating multiple data streams can be used in future studies to identify confounders in investigations of buildings, IAQ, and residents' health, so that a more comprehensive relationship between building performance, IAQ, environmental factors, and residents' health can be developed.

Our study quantified culturable fungi and bacteria concentrations, including their seasonal variability, in three multi-apartment residential buildings with different ventilation systems. These reports, along with guidelines and standards proposed by several international agencies (CEC, 1993; Moon et al., 2014; Rao et al., 1996), can facilitate the development of IAQ standards for bioaerosols in the US. A few of the guidelines and standards used for categorizing exposures are: Górny & Dutkiewicz (2002) have proposed 500 CFU/m³ as the residential limit values for airborne bacteria and fungi in European countries. The Commission of European Communities has categorized the culturable fungi concentrations in houses ranging from < 50 CFU/m³ to > 10^4 CFU/m³ as "very low" to "very high" levels, respectively (CEC, 1993). The S. Korean government has established that the concentration of airborne culturable bacteria in the indoor air of public use facilities should not exceed 800 CFU/m³ (Moon et al., 2014).

The presence of central heating, ventilation, and air-conditioning (HVAC) system led to lower indoor levels of culturable fungi; however, it controlled the indoor culturable bacteria to a lesser extent. We showed that lower dew points lead to lower concentrations of culturable fungal and bacterial aerosols. Understanding the effect of building infrastructure and environmental variables, such as dew point, on the presence of culturable bioaerosols could help residents lower their exposures to bioaerosols, thus minimizing the risk of allergic and respiratory illnesses. Our findings suggest that having a central HVAC system and lowering indoor dew point could decrease occupants' exposure to bioaerosols. Nonetheless, HVAC systems can increase the total energy consumption of buildings.

Our findings on the biological collection performances of personal samplers should be considered during the selection of a sampler for personal bioaerosol exposure assessments. Environmental sampling with filters and micro-centrifuge tubes measured higher bioaerosol number concentrations compared to liquid-based sampling. Whereas, the samples recovered from the electrostatics-based sampler (PEBS) had higher proportions of live cells and comparable culturable concentrations to the other personal samplers.

5.3 Potential Applications of PEBS

Existing stationary and portable bioaerosol samplers need separate and cumbersome sampling pumps, have high power consumption, are unable to operate for extended periods (in most cases), and have low sampling flow rates which result in poor detection limits. Standard filter samplers can cause desiccation of the captured bioaerosols and require an external power supply, while liquid samplers have been shown to have high latent internal losses and poor collection efficiency for small particles (T. Han & Mainelis, 2012). Passive aerosol sampling eliminates the need for the power supply but requires extended sampling periods and cannot be used for grab sampling or a full day work shift (Therkorn et al., 2017).

These challenges are overcome by the development of PEBS at Rutgers University. PEBS is a lightweight, battery-operated, self-contained sampler that has novel technology designed for high bioaerosol collection efficiency (T. T. Han et al., 2017, 2018). These features of PEBS, including its quiet operation and high sampling flow rate, make it superior to other commercially available and adapted personal bioaerosol samplers. The high sampling flow rate allows for both short and long term sampling. Short-term sampling and rapid lossless sample recovery provide unique opportunities to the greater scientific community in effective biosurveillance and formulation of necessary control measures. Long-term sampling allows researchers to determine exposure risks in occupational (4 - 8)

hours work period) and residential studies. It can also facilitate the development of doseresponse relationships for exposure to bioaerosols. Since PEBS has no size-selective inlet, additional analysis techniques can account for bioaerosol exposure assessments of virions. PEBS can also be utilized for gene sequencing of bioaerosols from different ecological areas and seasons. Speciation of ambient bioaerosols can be correlated with local health problems and environmental concerns and will provide information that is currently unavailable.

5.4 Future Directions

Future research directions will focus on further investigation of the associations between building structural deficiencies, IAQ, environmental parameters, and human health. There were several limitations observed during this research that should be addressed in future studies, and they are discussed below.

5.4.1 Detection of Building Structural Deficiencies in a Controlled Lab Study

A controlled lab study can advance our understanding of the mechanisms governing the relationship between the missing insulation and the presence of particles indoors. Different types of insulation, varied wall thickness, and outdoor environmental conditions should be considered. Larger studies and multiple buildings could show correlations of IAQ with other types of building deficiencies as well.

5.4.2 Personal Bioaerosol Exposure in Residential Buildings

Personal bioaerosol sampling can be complemented with area sampling in residential buildings to identify the difference between an individual's personal exposure and background levels. Viability analyses and sequencing of bioaerosols will determine their physiological states and abundance in the study apartments, respectively. Dominant species will be identified, and potential health effects of bioaerosols can be linked in epidemiological studies (Main, 2003). This information will benefit residents with allergic diseases and respiratory infections triggered by bioaerosols. Disease-causing agents can also be identified from the sequences and thereby initiate prevention practices at homes.

5.4.3 Ventilation Systems, Building Location, and Repeated Sampling

Simultaneous sampling of multi-apartment residential buildings with different ventilation systems can further explain the variability of indoor bioaerosols. A recent review paper also recommends sampling when mechanic ventilation systems are turned on and off for better comparison (Cox et al., 2019). Contributions of local bioaerosol sources to IAQ can also be considered, such as buildings located in metropolitan *vs.* rural areas. Repeated sampling in buildings can present information on temporal variations of aerosols and bioaerosols, and this data can be juxtaposed with occupant health concerns.

5.4.4 Questionnaire for Residents

The following questionnaire data could strengthen the associations between IAQ determinants and residents' health: 1) number of occupants including children in the apartments, 2) common cleaning and cooking frequencies and types, 3) presence of pets and their kinds, 4) indoor smoking and vaping frequencies and extent including information on commonly used smoking and vaping products and devices, 5) timeframe when residents lived in the apartment (years), 6) time spent daily at their apartments (hours/day). These results could identify potential indoor sources and formulate the necessary pollution mitigation strategies.

5.4.5 Resident Health Concerns

The primary health questions recorded during individual interviews were incidences of asthma and its prevalence in the last 12 months. Medical records and information about the use of medications could further strengthen associations between indoor pollutants and residents' health. Records of cardiopulmonary diseases, infections, and allergic reactions can be complemented with our findings to develop guidelines for IAQ improvement.

5.5 References

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APPENDIX A: SUPPORTING INFORMATION FOR CHAPTER 2



(Guo, 2016).

Table A-1. Thermal infrared deficiencies detected in Building 1 (n = 4) and 2 (n = 16) (Guo,2016).

Apartment	Missing	Missing	R-value	Temperature	Temperature
Code	Insulation	Insulation		Factor-	Factor-Air
	(area in f ²)	(area in		Thermal	Leakage
		percentage)		Bridge	
H1	13.33	6.88%	0.85	0.63	0.63
H2	0.70	0.13%	0.86	0.78	0.68
H3	30.01	12.71%	1.08	0.81	0.77
H4	29.70	7.12%	0.83	0.93	0.91

Apartment	Missing	Missing	R-value	Temperature	Temperature
Code	Insulation	Insulation		Factor-	Factor-Air
	(area in f ²)	(area in		Thermal	Leakage
		percentage)		Bridge	
H1	1.40	0.55%	0.53	0.62	0.52
H2	3.60	1.41%	0.67	0.73	0.68
H3	13.00	5.25%	1.97	0.63	0.62
H4	0.65	0.26%	0.90	0.91	0.73
H5	36.50	14.30%	0.30	0.68	0.42
H6	1.79	0.70%	1.21	0.82	0.54
H7	0.99	0.39%	0.54	0.71	0.63
H8	2.63	0.83%	0.85	0.62	0.35
H9	4.21	1.65%	1.13	0.95	0.68
H10	36.30	19.62%	0.31	0.75	0.49
H11	9.32	3.65%	4.06	1.06	1.05
H12	9.02	3.58%	2.01	0.94	0.84
H13	1.13	0.61%	1.52	0.88	0.83
H14	0.45	0.18%	2.70	0.90	0.68
H15	5.89	2.31%	1.68	0.87	0.71
H16	3.30	1.29%	2.09	0.89	0.77

Appendix A. Reference

 M. Guo, Spatially resolved infrared imaging for building performance evaluation, Rutgers University - Graduate School - New Brunswick, 2016. doi:10.7282/T3ZK5JSG.

APPENDIX B: SUPPORTING INFORMATION FOR CHAPTER 3

B-1. Culturable Bioaerosol Concentrations for the Three Campaigns of Building 1 *Concentrations in building campaigns without seasonal stratification*

The bioaerosol concentrations stratified by the seasons and campaigns are shown in Figure B-1 and Figure B-2. There was a significant effect of campaigns (B1-C1, B1-C2, and B1-C3) on culturable fungi concentrations ($\chi^2(2) = 18.462$, p < 0.001) and culturable bacteria I/O ratios ($\chi^2(2) = 4.815$, p = 0.045). However, culturable fungi I/O ratios ($\chi^2(2) = 1.090$, p = 0.580) and culturable bacteria concentrations ($\chi^2(2) = 2.221$, p = 0.329) did not differ between the campaigns.

Seasonal effect within each building campaign: Culturable fungi concentrations (Figure *B-1. a*)

B1-C1: Concentrations of culturable fungi differed in apartments of BI-C1 for the three seasons ($\chi^2(2) = 8.461$, p = 0.015), with the lowest median concentration measured in spring (median: 60 CFU/m³). The median concentration in summer (median: 142 CFU/m³) was 2x higher than the median concentration in spring (p = 0.008). There was no statistically significant difference between the concentrations in fall (median: 105 CFU/m³) and spring (p = 0.230), but a borderline significant difference was measured between the concentrations in fall and summer (p = 0.057).

B1-C2: Concentrations of culturable fungi differed in apartments of BI-C2 for the four seasons ($\chi^2(3) = 36.68$, p < 0.001). Winter measurements in B1-C2 had the lowest median concentration of 18 CFU/m³ compared to spring (median: 78 CFU/m³; p = 0.002), summer (median: 110 CFU/m³; p < 0.001) and fall (median: 57 CFU/m³; p > 0.05). Fall

concentrations did not differ significantly from those in other seasons (p > 0.1). Spring and summer concentrations were fairly similar to each other, differing only in their ranges.

B1-C3: Spring and winter concentrations in B1-C3 were similar to each other (U= 37.5, p = 0.225) with median values of 29 CFU/m³ and 32 CFU/m³, respectively.

Seasonal effect within each building campaign: Culturable fungi I/O ratio (Figure B-1. b)

B1-C1: Campaign 1 of Building 1 had a similar culturable fungi I/O ratios during summer (median: 0.61) and fall (median: 0.60) (p = 0.370). The median I/O ratio in spring (median: 0.32) was significantly lower and almost half the median I/O ratio of both summer (p = 0.034) and fall (p = 0.035) seasons.

B1-C2: B1-C2 had similar I/O ratios for all four seasons, with median values ranging from 0.35 to 0.62. Though the median I/O ratio in winter was the lowest compared to the other seasons, the 75th percentile of the winter I/O ratio value was the highest, with a value of 1.39.

B1-C3: The median I/O ratio in spring (median: 0.90) was more than twice that of winter (median: 0.38) (U = 23.0, p = 0.030).

Seasonal effect within each building campaign: Culturable bacteria concentrations (Figure B-2. a)

B1-C1: The median concentrations for spring, summer, and fall of B1-C1 were not significantly different from each other ($\chi^2(2) = 3.37$, p = 0.185), with values 235 CFU/m³, 437 CFU/m³, and 196 CFU/m³, respectively. A similar result was observed for the other two campaigns of Building 1.

B1-C2: The indoor median concentrations did not differ during the four seasons ($\chi^2(3) = 3.921$, p = 0.270).

B1-C3: The median concentrations in spring (median: 194 CFU/m³) and winter (median: 290 CFU/m³) did not differ significantly (U = 36.0, p = 0.407).

Seasonal effect within each building campaign: Culturable bacteria I/O ratio (Figure B-2. b)

B1-C1: The ratios during three seasons in B1-C1 were significantly different from each other ($\chi^2(2) = 9.45$, p = 0.009), with median values ranging from 0.85 to 2.27. Summer (median: 2.27) had the highest median I/O ratio and was borderline significantly higher than that in fall (median: 0.85) (p = 0.072). Spring had I/O ratios (median: 1.13) similar to both summer (p = 0.252) and fall (p = 0.927).

B1-C2: The I/O ratios differed between the seasons ($\chi^2(3) = 14.78$, p = 0.002), with the lowest median I/O ratio measured in winter (median: 0.76). The I/O ratios in spring, summer, and fall were similar to each other, with median values 0.99, 1.28, and 1.27, respectively. The I/O ratios in winter were borderline significantly lower than that in summer (p = 0.1).

B1-C3: Though spring had a higher median I/O ratio of 1.83, it was not significantly higher than that in winter (median: 1.18) (U = 42.0, p = 0.347).

Differences between building campaigns in individual seasons

Spring: The culturable fungi concentrations ($\chi^2(2) = 6.164$, p = 0.046) and it's I/O ratio ($\chi^2(2) = 10.579$, p = 0.005) differed significantly between the three campaigns. However,

the culturable bacteria concentrations ($\chi^2(2) = 1.117$, p = 0.572) and it's I/O ratio ($\chi^2(2) = 1.394$, p = 0.498) were similar.

Summer: Comparisons of the culturable bioaerosol concentrations between the two campaigns in summer juxtaposed to that of spring. The culturable fungi concentrations (U = 380.5; p = 0.457) and their I/O ratio (U = 365.5; p = 0.334) were similar between B1-C1 and B1-C2, while the culturable bacteria concentrations (U = 288.0; p = 0.034) and their I/O ratio (U = 250.0; p = 0.007) differed significantly between the two campaigns.

Fall: The differences of the indoor culturable bioaerosol concentrations and their I/O ratios were similar to the result of the indoor concentrations in building campaigns without seasonal stratification. The concentrations differed significantly between the B1-C1 and B1-C2 campaigns for culturable fungi concentrations (U = 251.0; p = 0.062) and culturable bacteria I/O ratios (U = 266.0; p = 0.096), with borderline significance. However, culturable fungi I/O ratios (U = 282.0; p = 0.312) and culturable bacteria concentrations (U = 314.0; p = 0.650) did not differ between the two campaigns.

Winter: B1-C3 had significantly higher concentrations than B1-C2 for culturable mold (U = 169.0; p = 0.038) and bacteria (U = 188.0; p = 0.089) concentrations, and culturable bacteria I/O ratios (U = 181.5; p = 0.068). However, the culturable mold I/O ratios were similar between the two campaigns (U = 245.5; p = 0.955).

B-2. Association of Culturable Biaerosols with Indoor Dew Points

The association of indoor culturable fungi and bacteria concentrations stratified by apartments with different types of ventilation systems for the four seasons are shown in Figure B-4 and Figure B-5, respectively. The seasons are color-coded as green, yellow, orange, and blue representing spring (n = 109), summer (n = 129), fall (n = 88), and winter (n = 81), respectively. The "central HVAC" apartment data are exclusively from B2 as it has a central HVAC, while the "window AC" apartments are data pooled from B1 and B3. For the "central HVAC" apartments, the indoor culturable fungi concentrations had a moderate yet significant positive association with indoor DP in spring (r_s = 0.586; p < 0.001) and winter (r_s = 0.366; p = 0.015). In summer (r_s = 0.288; p = 0.018) and fall (r_s = 0.251; p = 0.086), a weak yet significantly or bordeline positive association was observed. For indoor culturable bacteria concentrations and indoor DP in the "central HVAC" apartments, a moderate and weak yet significantly positive association was observed for fall (r_s = 0.352; p = 0.026) and winter (r_s = 0.284; p = 0.049). However, spring (r_s = -0.121; p = 0.228) and summer (r_s = -0.046; p = 0.372) did not have a significant associations between indoor culturable bacteria and indoor DP for the "central HVAC" apartments.

For the "window AC" apartments, the indoor culturable fungi concentrations had a strong to moderate yet significantly positive association with indoor DPs in spring ($r_s = 0.660$; p < 0.001), fall ($r_s = 0.427$; p < 0.001), and winter ($r_s = 0.451$; p < 0.001). However, there were no associations between the two variables in summer ($r_s = 0.022$; p = 0.425). Indoor culturable bacteria concentrations and indoor DP had a weak yet significant positive association in spring ($r_s = 0.325$; p = 0.003) and winter ($r_s = 0.263$; p = 0.038) in the "window AC" apartments. However, in summer ($r_s = 0.072$; p = 0.268) and fall ($r_s = 0.050$; p = 0.355), there was no significant association between indoor culturable bacteria concentrations and indoor DP.



b)



Figure B-1. Measurements of a) indoor culturable fungi concentration (CFU/m³) and b) culturable fungi indoor-outdoor ratio stratified by the three campaigns of building 1 (B1-C1, B1-C2, and B1-C3) and by four seasons (Spring, Summer, Fall, and Winter). The dotted red line represents the indoor/outdoor ratio equal to 1. The upward-facing triangles and downward facing triangles represent 1st and 99th percentile of the data, respectively; the whiskers represent 1.5x of interquartile range; the square represents the mean; the lower, middle and upper lines of the box plot are 25th percentile, median, and 75th percentile of the data, respectively. * represents a group(s) that are significantly different with a p-value less than 0.05.



b)



Figure B-2. Measurements of a) indoor culturable bacteria concentration (CFU/m³) and b) culturable bacteria indoor-outdoor ratio stratified by the three campaigns of building 1 (B1-C1, B1-C2, and B1-C3) and by four seasons (Spring, Summer, Fall, and Winter). The dotted red line represents the indoor/outdoor ratio equal to 1. The upward-facing triangles and downward facing triangles represent 1^{st} and 99^{th} percentile of the data, respectively; the whiskers represent 1.5x of interquartile range; the square represents the mean; the lower, middle and upper lines of the box plot are 25^{th} percentile, median, and 75^{th} percentile of the data, respectively. [#] represents a group(s) that are significantly different with a p-value of less than 0.1.



b)



Figure B-3. a) Outdoor dew point (°F) and b) outdoor temperature (°F) stratified by seasons (Spring, Summer, Fall, and Winter) for the three buildings (B1, B2, B3). The upward-facing triangles and downward-facing triangles represent 1st and 99th percentile of the data, respectively; the whiskers represent 1.5x of interquartile range; the black square represents the mean; the lower, middle and upper lines of the box plot are 25th percentile, median, and 75th percentile of the data, respectively. The asterisk (*) represents a statistically significant difference (p < 0.05) between the groups.



Figure B-4. Association of indoor dew point (°F) with indoor culturable fungi concentrations (CFU/m³) measured in building(s) stratified by central Heating Ventilating and Cooling system ("central HVAC" apartments; B2) and with radiator heat and window air-conditioning (AC) units ("window AC" apartments; B1 and B3) for four seasons color-coded as green, yellow, orange, and blue representing spring, summer, fall, and winter, respectively. Correlation analysis was given by Spearman correlation coefficient (r_s) along with the p-value; n represents the number of samples for each season; the trendline represents the line of best fit.



Indoor Dew Point (°F)
Figure B-5. Association of indoor dew point (°F) with indoor culturable bacteria concentrations (CFU/m³) measured in building(s) stratified by central Heating Ventilating and Cooling system ("central HVAC" apartments; B2) and with radiator heat and window air-conditioning (AC) units ("window AC" apartments; B1 and B3) for four seasons color-coded as green, yellow, orange, and blue representing spring, summer, fall, and winter, respectively. Correlation analysis was given by Spearman correlation coefficient (r_s) along with the p-value; n represents the number of samples for each season; the trendline represents the line of best fit.

APPENDIX C: SUPPORTING INFORMATION FOR CHAPTER 4

Table C-1. Total bioaerosol number concentrations measured by five personal samplers at three sites (Site 1: Horse barn; Site 2: Outdoors; Site 3: Greenhouse) as determined using epifluorescence microscopy with acridine orange staining and direct microscopy with a hemocytometer for bacteria and fungi, respectively. Values are shown as average \pm standard deviation of 4 repeats per sampler (2 samples per day on two consecutive days). Each repeat was analyzed in triplicates. The bacteria + fungi number concentration is the average value of the sum of number concentrations of bacteria and fungi for each repeat.

Number	Button	PEBS	CIP	UPAS	NIOSH-L	NIOSH-S	NIOSH-F	NIOSH-T
concentration (#/m ³)			10-M					
			SITE 1 (r	n = 4 for ea	ch sampler)			
Bacteria	(4.12 ±	(2.26 ±	(1.82 ±	(3.28 ±	(2.84 ±	(2.02 ±	(3.92 ±	(5.26 ±
	0.58)	0.86)	1.09)	1.84)	1.54)	1.65)	1.32)	3.20)
	x10 ⁶	x10 ⁵	x10 ⁶					
Fungi	(7.16 ±	(3.13 ±	(3.18 ±	$(8.85 \pm$	(7.59 ±	(3.13 ±	(2.38 ±	(1.31 ±
	1.07)	0.45)	0.91)	3.13)	1.57)	0.29)	0.49)	0.18)
	x10 ⁴	x 10 ⁴	x10 ³	x10 ⁵				
Bacteria +	(4.19 ±	(2.29 ±	(1.85 ±	(3.37 ±	(2.92 ±	(2.05 ±	(4.16 ±	(5.39 ±
Fungi	0.59)	0.86)	1.09)	1.83)	1.55)	1.65)	1.34)	3.21)
	x10 ⁶	x10 ⁵	x10 ⁶					
			SITE 2 (r	n = 4 for ea	ch sampler)			

Bacteria	(1.16±	(7.22 ±	(3.98 ±	(1.71 ±	(1.42 ±	(1.17 ±	(2.13 ±	(2.80 ±
	0.27)	2.34)	1.20)	0.76)	0.38)	0.28)	0.71)	0.59)
	x10 ⁶	x10 ⁵	x10 ⁵	x10 ⁶	x10 ⁶	x10 ⁶	x10 ⁵	x10 ⁶
Fungi	(7.03 ±	$(3.80 \pm$	$(2.86 \pm$	(1.51 ±	(7.59 ±	(5.95 ±	(2.38 ±	(1.59 ±
	1.24)	1.71)	0.82)	0.49)	2.93)	0.49)	0.49)	0.31)
	x10 ⁴	x10 ⁴	x10 ⁴	x10 ⁵	x10 ⁴	x10 ⁴	x10 ⁴	x10 ⁵
Bacteria +	(1.23 +	(7.60 +	(4.26 +	(1.87 +	(1.50 +	(1.23 +	(2.37 +	(2.96 +
Fungi	0.26)	2 20)	1.26)	(1.07 ±	(1.50 ±	0.28)	0.75)	(2.90 ±
	0.20)	2.20)	1.20)	0.38)	0.38)	0.28)	0.75)	0.39)
	x10 ⁶	x10 ⁵	x10 ⁵	x10 ⁶	x10 ⁶	x10 ⁶	x10 ⁵	x10 ⁶
			SITE 3 (1	n = 4 for ea	ch sampler)			
Bacteria	(1.07 ±	(6.32 ±	(3.15 ±	(1.68 ±	(8.79 ±	(6.57 ±	(1.74 ±	(1.71 ±
Bacteria	(1.07 ± 0.44)	(6.32 ± 1.21)	(3.15 ± 0.51)	(1.68 ± 0.37)	(8.79 ± 1.78)	(6.57 ± 0.75)	(1.74 ± 0.39)	(1.71 ± 0.13)
Bacteria	(1.07 ± 0.44) x10 ⁶	(6.32 ± 1.21) x10 ⁵	(3.15 ± 0.51) $x10^{5}$	(1.68 ± 0.37) $x10^{6}$	(8.79 ± 1.78) x10 ⁵	(6.57 ± 0.75) $x10^5$	(1.74 ± 0.39) x10 ⁵	(1.71 ± 0.13) x10 ⁶
Bacteria	(1.07 ± 0.44) x10 ⁶	(6.32 ± 1.21) x10 ⁵	(3.15 ± 0.51) $x10^{5}$	(1.68 ± 0.37) x10 ⁶	(8.79 ± 1.78) x10 ⁵	(6.57 ± 0.75) x10 ⁵	(1.74 ± 0.39) x10 ⁵	(1.71 ± 0.13) x10 ⁶
Bacteria Fungi	(1.07 ± 0.44) $x10^{6}$ $(2.47 \pm$	(6.32 ± 1.21) $x10^{5}$ $(1.93 \pm$	(3.15 ± 0.51) $x10^{5}$ $(1.15 \pm$	(1.68 ± 0.37) $x10^{6}$ $(8.33 \pm$	(8.79 ± 1.78) $x10^{5}$ $(3.13 \pm$	(6.57 ± 0.75) $x10^{5}$ $(1.93 \pm$	(1.74 ± 0.39) $x10^{5}$ $(1.93 \pm$	(1.71 ± 0.13) $x10^{6}$ $(6.99 \pm$
Bacteria Fungi	(1.07 ± 0.44) x10 ⁶ (2.47 ± 1.37)	(6.32 ± 1.21) $x10^{5}$ (1.93 ± 0.26)	(3.15 ± 0.51) $x10^5$ (1.15 ± 0.27)	(1.68 ± 0.37) $x10^{6}$ (8.33 ± 1.70)	(8.79 ± 1.78) $x10^5$ (3.13 ± 0.75)	(6.57 ± 0.75) $x10^5$ (1.93 ± 0.89)	(1.74 ± 0.39) $x10^{5}$ (1.93 ± 0.57)	(1.71 ± 0.13) $x10^{6}$ (6.99 ± 1.64)
Bacteria Fungi	(1.07 ± 0.44) $x10^{6}$ (2.47 ± 1.37) $x10^{4}$	(6.32 ± 1.21) $x10^{5}$ (1.93 ± 0.26) $x10^{4}$	(3.15 ± 0.51) $x10^{5}$ (1.15 ± 0.27) $x10^{4}$	(1.68 ± 0.37) $x10^{6}$ (8.33 ± 1.70) $x10^{4}$	(8.79 ± 1.78) $x10^{5}$ (3.13 ± 0.75) $x10^{4}$	(6.57 ± 0.75) $x10^{5}$ (1.93 ± 0.89) $x10^{4}$	(1.74 ± 0.39) $x10^{5}$ (1.93 ± 0.57) $x10^{4}$	(1.71 ± 0.13) x10 ⁶ (6.99 ± 1.64) x10 ⁴
Bacteria Fungi	(1.07 ± 0.44) $x10^{6}$ (2.47 ± 1.37) $x10^{4}$	(6.32 ± 1.21) $x10^{5}$ (1.93 ± 0.26) $x10^{4}$	(3.15 ± 0.51) $x10^{5}$ (1.15 ± 0.27) $x10^{4}$	(1.68 ± 0.37) $x10^{6}$ (8.33 ± 1.70) $x10^{4}$	(8.79 ± 1.78) $x10^{5}$ (3.13 ± 0.75) $x10^{4}$	(6.57 ± 0.75) $x10^5$ (1.93 ± 0.89) $x10^4$	(1.74 ± 0.39) $x10^{5}$ (1.93 ± 0.57) $x10^{4}$	(1.71 ± 0.13) $x10^{6}$ (6.99 ± 1.64) $x10^{4}$
Bacteria Fungi Bacteria +	(1.07 ± 0.44) x10 ⁶ (2.47 ± 1.37) x10 ⁴ (1.13 ± 1.100)	(6.32 ± 1.21) $x10^{5}$ (1.93 ± 0.26) $x10^{4}$ (6.51 ± 10^{4})	(3.15 ± 0.51) $x10^{5}$ (1.15 ± 0.27) $x10^{4}$ (3.26 ± 0.27)	(1.68 ± 0.37) $x10^{6}$ (8.33 ± 1.70) $x10^{4}$ $(1.77 \pm)$	(8.79 ± 1.78) $x10^{5}$ (3.13 ± 0.75) $x10^{4}$ (9.10 ± 10^{4})	(6.57 ± 0.75) $x10^5$ (1.93 ± 0.89) $x10^4$ (6.76 ± 0.75)	(1.74 ± 0.39) x10 ⁵ (1.93 ± 0.57) x10 ⁴ (1.93 ± 0.57)	(1.71 ± 0.13) x10 ⁶ (6.99 ± 1.64) x10 ⁴ (1.78 ± 0.12)
Bacteria Fungi Bacteria + Fungi	(1.07 ± 0.44) $x10^{6}$ (2.47 ± 1.37) $x10^{4}$ (1.13 ± 0.43)	(6.32 ± 1.21) $x10^{5}$ (1.93 ± 0.26) $x10^{4}$ (6.51 ± 1.23)	(3.15 ± 0.51) $x10^{5}$ (1.15 ± 0.27) $x10^{4}$ (3.26 ± 0.52)	(1.68 ± 0.37) x10 ⁶ (8.33 ± 1.70) x10 ⁴ (1.77 ± 0.38)	(8.79 ± 1.78) $x10^{5}$ (3.13 ± 0.75) $x10^{4}$ (9.10 ± 1.81)	(6.57 ± 0.75) $x10^{5}$ (1.93 ± 0.89) $x10^{4}$ (6.76 ± 0.76)	(1.74 ± 0.39) $x10^{5}$ (1.93 ± 0.57) $x10^{4}$ (1.93 ± 0.35)	(1.71 ± 0.13) x10 ⁶ (6.99 ± 1.64) x10 ⁴ (1.78 ± 0.15)
Bacteria Fungi Bacteria + Fungi	(1.07 ± 0.44) x10 ⁶ (2.47 ± 1.37) x10 ⁴ (1.13 ± 0.43) x10 ⁶	$(6.32 \pm 1.21) \\ x10^{5} \\ (1.93 \pm 0.26) \\ x10^{4} \\ (6.51 \pm 1.23) \\ x10^{5} \\ \end{cases}$	(3.15 ± 0.51) $x10^{5}$ (1.15 ± 0.27) $x10^{4}$ (3.26 ± 0.52) $x10^{5}$	(1.68 ± 0.37) $x10^{6}$ (8.33 ± 1.70) $x10^{4}$ (1.77 ± 0.38) $x10^{6}$	(8.79 ± 1.78) $x10^{5}$ (3.13 ± 0.75) $x10^{4}$ (9.10 ± 1.81) $x10^{5}$	(6.57 ± 0.75) $x10^{5}$ (1.93 ± 0.89) $x10^{4}$ (6.76 ± 0.76) $x10^{5}$	(1.74 ± 0.39) x10 ⁵ (1.93 ± 0.57) x10 ⁴ (1.93 ± 0.35) x10 ⁵	(1.71 ± 0.13) x10 ⁶ (6.99 ± 1.64) x10 ⁴ (1.78 ± 0.15) x10 ⁶

Table C-2. Adenosine triphosphate (ATP) concentrations in Relative Luminescence Units (RLU/m^3) for five personal samplers at three sites (Site 1: Horse barn; Site 2: Outdoors; Site 3: Greenhouse). Values are presented as average ± standard deviation of 4 repeats per sampler (2 samples per day on two consecutive days). Each repeat was analyzed in triplicates.

ATP	Button	PEBS	CIP 10-	UPAS	NIOSH-	NIOSH-	NIOSH-	NIOSH-
concentration			М		L	S	F	Т
(RLU/m ³)								
Site 1	(2.96 ±	(2.56 ±	(3.79 ±	(2.66 ±	(7.94 ±	(3.19 ±	(3.43 ±	(1.15 ±
	1.23)	1.11)	0.36)	1.52)	2.55)	2.67)	0.23)	0.52)
	x10 ⁵	x10 ⁴	x10 ⁶					
Site 2	(4.31 ±	(5.93 ±	(4.91 ±	(1.14 ±	(7.24 ±	(3.59 ±	(9.37 ±	(1.18 ±
	1.94)	0.41)	0.78)	0.61)	1.36)	1.46)	6.57)	0.32)
	x10 ⁴	x10 ⁴	x10 ⁴	x10 ⁵	x10 ⁴	x10 ⁴	x10 ³	x10 ⁵
Site 3	(2.09 ±	(2.67 ±	(2.15 ±	(1.11 ±	(3.24 ±	(2.83 ±	(1.99 ±	(6.26 ±
	1.07)	0.40)	0.23)	0.35)	1.11)	0.50)	1.23)	0.72)
	x10 ⁵	x10 ⁴	x10 ⁵					

Table C-3. Proportions of live cells (in percentage, %) for five personal samplers at three sites (Site 1: Horse barn; Site 2: Outdoors; Site 3: Greenhouse). Values are presented as average \pm standard deviation of 4 repeats per sampler (2 samples per day on two consecutive days). Each repeat was analyzed in triplicates.

Live	Button	PEBS	CIP	UPAS	NIOSH-	NIOSH-	NIOSH-
cells			10-M		L	S	\mathbf{F}
(%)							
Site 1	26.78 ±	$37.98 \pm$	$13.88 \pm$	25.03 ±	26.43 ±	25.95 ±	$27.05 \pm$
	7.20	5.53	8.64	1.53	1.93	3.94	2.50
Site 2	16.63 ±	32.33 ±	$19.00 \pm$	$18.48 \pm$	17.43 ±	17.63 ±	19.68 ±
	1.78	3.67	1.33	0.94	1.61	1.93	2.22
Site 3	24.43 ±	41.98±	$26.53 \pm$	$24.75 \pm$	$27.08 \pm$	14.63 ±	$13.05 \pm$
	10.40	12.01	4.12	5.94	2.30	2.40	1.77

Table C-4. Proportions of dead cells (in percentage, %) for five personal samplers at three sites (Site 1: Horse barn; Site 2: Outdoors; Site 3: Greenhouse). Values are presented as average \pm standard deviation of 4 repeats per sampler (2 samples per day on two consecutive days). Each repeat was analyzed in triplicates.

Dead	Button	PEBS	CIP	UPAS	NIOSH-	NIOSH-	NIOSH-
cells			10-M		L	S	F
(%)							
Site 1	11.93 ±	$4.55 \pm$	$23.05 \pm$	11.33 ±	13.53 ±	15.65 ±	14.23 ±
	6.25	0.75	10.46	3.62	3.34	2.68	1.64
Site 2	19.45 ±	6.93 ±	$16.58 \pm$	18.65 ±	18.35 ±	$18.48 \pm$	$14.50 \pm$
	4.64	3.85	1.32	2.03	2.60	5.17	9.90
Site 3	14.95 ±	$8.00 \pm$	13.00 ±	15.68 ±	13.10 ±	21.45 ±	21.55 ±
	6.31	5.53	2.51	3.10	1.18	0.95	3.19

Table C-5. Total bioaerosol culturable concentrations measured by five personal samplers at three sites (Site 1: Horse barn; Site 2: Outdoors; Site 3: Greenhouse) as determined using agar plating with Trypticase soy agar (TSA) and malt extract agar (MEA) for bacteria and fungi, respectively. Values are shown as average \pm standard deviation of 4 repeats per sampler (2 samples per day on two consecutive days). Each repeat was analyzed in triplicates. The bacteria + fungi number concentration is the average value of the sum of number concentrations of bacteria and fungi for each repeat.

Culturable	Button	PEBS	CIP 10-	UPA-	NIOSH-L	NIOSH-S	NIOSH-F	NIOSH-T
concentration (CFU/m ³)			Μ	S				
		SIT	E 1 (n = 4)	4 for ea	ch sample	er)		
Bacteria	6198	3260	17478	608	11917	300	60	12277
	±	±	±	±	±	±	±	±
	748	138	1552	622	4461	45	54	4447
Fungi	595	306	43	521	345	159	119	583
	±	±	±	±	±	±	±	±
	284	177	38	127	117	69	73	202
Bacteria +	6793	35662	1977	1128	12262	459	179	12860
Fungi	±	±	±	±	±	±	±	±
	947	1285	1523	662	4404	74	116	4308
		SIT	E 2 (n = -4	4 for ea	ich sample	er)		

Bacteria		443	186	122	278	134	40	0	174
		±	±	±	±	±	±		±
		553	45	92	372	110	67		113
Fungi		78	31	43	208	124	104	40	268
		±	±	±	±	±	±	±	±
		64	14	38	127	123	127	43	272
Bacteria	+	521	217	165	486	258	144	40	441
Fungi		±	±	±	±	<u>+</u>	±	±	±
		526	51	100	471	106	188	43	289
			SITI	E 3 (n = -)	4 for eac	ch sample	er)		
Bacteria		48	76	54	52	107	25	0	131
Bacteria		48 ±	76 ±	54 ±	52 ±	107 ±	25 ±	0	131 ±
Bacteria		48 ± 30	76 ± 95	54 ± 32	52 ± 35	107 ± 74	25 ± 30	0	131 ± 98
Bacteria Fungi		48 ± 30 464	76 ± 95 89	54 ± 32 314	52 ± 35 226	107 ± 74 174	25 ± 30 89	0 55	131 ± 98 317
Bacteria Fungi		48 ± 30 464 ±	76 ± 95 89 ±	54 ± 32 314 ±	52 ± 35 226 ±	107 ± 74 174 ±	25 ± 30 89 ±	0 55 ±	131 ± 98 317 ±
Bacteria Fungi		48 ± 30 464 ± 227	76 ± 95 89 ± 36	54 ± 32 314 ± 270	52 ± 35 226 ± 183	107 \pm 74 174 \pm 55	25 ± 30 89 ± 34	0 55 ± 19	131 ± 98 317 ± 32
Bacteria Fungi Bacteria	+	$ \begin{array}{c} 48 \\ \pm \\ 30 \\ 464 \\ \pm \\ 227 \\ 512 \\ \end{array} $	76 ± 95 89 ± 36 165	54 ± 32 314 ± 270 368	52 ± 35 226 ± 183 278	107 \pm 74 174 \pm 55 280	25 ± 30 89 ± 34 114	0 55 ± 19 55	$ \begin{array}{c} 131 \\ \pm \\ 98 \\ 317 \\ \pm \\ 32 \\ 449 \end{array} $
Bacteria Fungi Bacteria Fungi	+	$ \begin{array}{c} 48 \\ \pm \\ 30 \\ 464 \\ \pm \\ 227 \\ 512 \\ \pm \\ \end{array} $	76 ± 95 89 ± 36 165 ±	54 ± 32 314 ± 270 368 ±	52 ± 35 226 ± 183 278 ±	107 \pm 74 174 \pm 55 280 \pm	25 ± 30 89 ± 34 114 ±	0 55 ± 19 55 ±	131 ± 98 317 ± 32 449 ±