Graduate School Form 9
(Revised 6/03)

PURDUE UNIVERSITY
GRADUATE SCHOOL
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Entitled Detection of Indoor Airborne Fungal Contamination through Examination of Building Heating, Ventilating and Air Conditioning (HVAC) Filters

Complies with University regulations and meets the standards of the Graduate School for originality and quality

For the degree of Doctor of Philosophy

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DETECTION OF INDOOR AIRBORNE FUNGAL CONTAMINATION THROUGH EXAMINATION OF BUILDING HEATING, VENTILATING AND AIR CONDITIONING (HVAC) FILTERS

A Thesis

Submitted to the Faculty

of

Purdue University

by

Hernando R. Perez

In Partial Fulfillment of the

Requirements for the Degree

of

Doctor of Philosophy

August 2004

To my parents Rafael Guillermo Perez Gonzalez and Rosario Perez. Thank you for your guidance. Without your love and support none of my accomplishments in life would have been possible

ACKNOWLEDGEMENTS

There are many persons without whom this work could not have been completed and I am grateful to have this opportunity to express my thanks to them. I would like to express my deepest thanks to my major advisor, Dr. Neil Zimmerman for his unwavering and patience and support over the last four years. His mentoring, encouragement, and guidance during my time at Purdue University have been an inspiration without which I could not have completed this degree. I would also like to thank Dr. Jin-Rong Xu for so generously providing his time and knowledge when I needed it most. My sincere thanks also go to Dr. Herman Cember and Dr. Frank Rosenthal for their insight, direction and time.

I would like to thank Dr. Lila Albin for her assistance in finding filters to work with early on in my research and Dr. Yan Chen for his expertise in helping to design my experimental chamber.

Finally, I thank my wife Valerie for not only being so patient and loving during the completion of this degree but also for providing so much assistance in the laboratory during the collection of my data.

This research study was supported by the NIOSH Pilot Research Training Program of the University of Cincinnati Education and Research Center Grant #T42/CCT510420.

TABLE OF CONTENTS

Page

Page

Page

LIST OF TABLES

Table Page

Table Page

LIST OF FIGURES

Figure Page

ABSTRACT

Perez, Hernando R. Ph.D., Purdue University, August 2004. Detection of Indoor Airborne Fungal Contamination Through Examination of Building Heating, Ventilating and Air Conditioning (HVAC) Filters. Major Professor: Neil J. Zimmerman.

This three phase research involved the pilot testing, laboratory development and field evaluation of a method for the quantification of viable fungal particles on heating, ventilating and air conditioning (HVAC) system filters. The primary purpose of this three phase work was to evaluate whether or not the airborne concentration of viable fungal particles calculated through the quantification of building HVAC filters is significantly associated with the average airborne viable fungal concentration as calculated through the collection of multiple single stage viable impactor samples taken at regular intervals while filters are in service. A second purpose of this research was to evaluate whether not the filter quantification method is able to differentiate, with respect to viable fungal airborne levels, between areas suspected of having significantly different concentrations. The filter quantification method involved the immersion of filter samples in 0.9% sterile saline, the shaking of the filter/saline combination, and the plating of aliquots of the shaking solution onto solid growth media. The inoculated media plates were incubated at room temperature for 96 hours at which time colonies were counted. The initial pilot phase of this research involved a comparison between complaint and

non-complaint university building HVAC filters. The results of the comparison indicated a statistically significant greater number of mold spores on the complaint filters than on the non-complaint filters when the results were normalized for airflow. The second research phase involved the use of a ventilation test chamber in which test filters were loaded with aerosolized *A. niger* or *P. chrysogenum* fungal spore suspensions before being processed as described above. Fungal recovery values as high as 93% were found with this method. In some cases recovery values of greater than 100% were obtained. The third phase of this research involved the comparison of filter quantification and single stage impactor results in several buildings. A statistically significant relationship between the two sampling procedures was found at both shorter and longer term sampling periods. Also in this phase, the filter quantification method was found to be more likely than impactor sampling to differentiate between areas with respect to airborne fungal concentrations.

CHAPTER I

INTRODUCTION

Purpose and Objectives

The purpose of research presented in this dissertation is to determine whether or not heating, ventilating, and air conditioning (HVAC) filter examination can be used as a means of evaluating relative levels of indoor airborne fungal contamination.

The objectives of this research are to:

1) To detect and quantify fungal contamination on/in building HVAC filters.
2) To compare HVAC filter concentrations and traditional sampling results between complaint and non-complaint areas of a building.
3) To validate a method for the quantification of fungi on HVAC filters using an experimental ventilation chamber.
4) To compare fungal concentrations detected on building HVAC filters with a traditional fungal sampling method.

Hypothesis

The quantification of viable fungal particles on HVAC filter materials can serve as an indicator of the relative level of viable particles in the air that the filter has handled.

Background

Indoor air has long been a source of exposure to substances that can cause adverse health effects in human occupants of buildings. Buildings provide shelter from the elements and allow for the development of a comfortable atmosphere where people come together to interact productively and efficiently. Due to the enclosed nature of buildings, there is the potential for contaminants to accumulate and become concentrated in the air. These contaminants could enter the building from outdoors or they could originate inside the building. Bearg[1] lists four key elements that interact in a building to yield the conditions of the indoor environment. These are the building shell, the heating, ventilating and air conditioning (HVAC) system and its condition, the outdoor environment, and the building occupants and their activities.

Ventilation is of key importance in maintaining indoor air quality (IAQ). Poor IAQ results when the quantity of ventilation air is insufficient to keep contaminant concentrations below levels that produce occupant health problems [1,2]. In 1989, the National Institute for Occupational Safety and Health (NIOSH) [4] reported the results of their survey of 529 buildings with IAQ complaints and found that in 53% of the buildings the IAQ problem was determined to be due to inadequate ventilation.

IAQ has received more attention since the early 1970's for two reasons. First, the general public became concerned because of the presence of friable asbestos in public

schools and other buildings. Second, the energy crisis of that time indirectly exacerbated the problem[3]. In an attempt to save energy, buildings were more tightly insulated. As a result less outdoor air was introduced into the buildings and contaminants built up.

The general focus of this project was the issue of indoor microbial contamination. The specific focus was on the correlation of indicators of fungal contamination with fungal presence and levels as measured through air sampling and examination of HVAC filters. Indicators of fungal contamination were determined through facility walkthroughs and histories.

The Environmental Protection Agency (EPA)[5] fits indoor pollutants into five major categories, which are summarized in Table 1.1. As is evident from this table, fungal problems can arise due to a number of events and circumstances. Sources outside the building include outdoor air with higher than normal levels of fungal spores. Equipment sources include standing water from improperly maintained HVAC drip pans. Human activities include things as simple as the keeping of keeping plants. Building materials include things such as wallpaper or wooden furnishings that can serve as nutrients and amplification sites for fungi. Other sources include accidental events such as floods that can lead to a great deal of fungal contamination.

Table 1.1. EPA Classification of Indoor Air Pollutant Sources

I. Sources Outside Building

A. contaminated outdoor air
- ex. Pollen, dust, fungal spores, general vehicle exhaust

B. Emissions form nearby sources
- ex. loading docks, odors from dumpsters, exhaust from nearby vehicles

C. Soil Gas
- ex. radon, leakage from under ground fuel tanks, pesticides

D. Moisture or standing water promoting excess microbial growth
- ex. Rooftops after rainfall, crawlspace

II. Equipment

A. HVAC system
- 1 ex. Dust or dirt in ductwork, microbial growth in drip pans, refrigerant leakage,

B. Non-HVAC equipment
- ex. Emissions from office equipment, solvents,toners, ammonia

III. Human Activities

A. Personal activities
- ex. Smoking, cooking, body odor, cosmetic odors

B. Housekeeping activities
- ex. Cleaning materials, deodorizers and fragrances, airborne dust

IV. Building Components and Furnishings

A. Locations that produce or collect dust or fibers
- ex open shelving, old furnishings, carpeting, curtains

B. Unsanitary conditions and water damage
- ex. Microbial growth on or in soiled or water damaged furnishings, dry traps that allow passage of sewer gas

C. Chemicals released from building components or furnishings
- ex. Volatile organic compounds or inorganic compounds

V. Other sources

A. Accidental events
- ex. Spills of water, flooding, fire damage

C. Special use areas and mixed use buildings
- ex. Smoking lounges, laboratories, print shops, beauty salons

D. Redecorating/Remodeling/Repair Activities
- emissions from new furnishings, dust and fibers from demolition, paint, caulk, adhesives

Fungi

Fungi are ubiquitous in the environment and account for approximately 25% of the earth's biomass[6]. They are eukaryotic organisms with rigid cell walls most often formed of chitin and glucans. Fungi include yeasts, molds, mildews, mushrooms, puffballs, and bracket fungi. Fungi can exist as single cells (yeasts), but more often exist as hyphae[7]. Hyphae are microscopic branched filaments, which can intertwine and form a mass called a mycelium. The mycelial fungi that are most commonly found indoors are often referred to as molds[8]. Mushrooms and brackets are examples of outdoor reproductive structures (mycelia) formed from the intertwining of hyphae.

Fungi are heterotrophic organisms and as such depend on external sources for nutrients. These sources are generally carbon compounds manufactured by other organisms. Fungi secrete enzymes that break down the nutrients, which are then absorbed through their cell walls. Fungi can be parasitic or symbiotic, but most are saprophytic, obtaining their nutrients from non-living organic matter.

Evolution has provided fungi with enzymes that can digest a great number of substrates, including many which are not digestible by the majority of other known organisms. These substrates include chitin, keratin, cellulose and lignin among others [9]. Some common indoor nutrient sources for fungi include starchy pastes used with wallpaper, cellulose in paper and fabrics, animal skin scales, wood, soap film on shower walls and plant soil [7,8]. Fungi are opportunistic organisms. Even a trace of moisture can lead to fungal spores colonizing a surface, whether that surface is food, fabric, paper or any other organic matter [9]. Because fungal spores are everywhere in our environment, unwanted mold contamination in buildings is not an uncommon phenomena.

Three key environmental factors affecting fungal survival and growth are water, temperature and nutrient availability. Fungi have an absolute requirement for water, but are very tolerant to wide ranges of water availability [7]. Water activity (a_w) is a term that is often used by mycologists to describe the amount of water within a substrate that an organism can use to support growth [8]. Water activity represents the ratio of water vapor pressure of the substrate to the water vapor pressure of pure water under the same environmental conditions. Kendrick [9] defines water activity as follows: the available water in a substrate as a decimal fraction of the amount present when the substrate is in equilibrium with a water vapor saturated atmosphere. For example, an equilibrium relative humidity of 70% around the substrate corresponds to an a_w of 0.70. Equilibrium relative humidity is the relative humidity that a substrate produces if enclosed with air in a sealed container at a constant temperature. Water activity is measured by allowing material to equilibrate in a sealed container and then measuring the relative humidity of the atmosphere in the container [8]. Water activity has a tendency to increase with increasing temperature.

Most bacteria will grow only at an a_w of 0.95 or higher. In contrast, many fungi can grow down to an a_w of 0.7 [7] meaning that these organisms have the ability to reproduce under relatively low levels of available water.

In addition to temperature's effect on a_w, temperature affects fungal growth directly [8]. Fungi can generally be categorized into three groups with respect to temperature, psychrophiles, mesophiles and thermophiles. There are general guidelines for categorizing fungi into one of these three groups. Most fungi are mesophilic, with an optimum growth temperature range between 15 and 40 °C, a minimum growth

temperature above 0°C and a maximum growth temperature below 50°C. Psychrophilic fungi have an optimum growth temperature range between 0 and 17°C, a minimum growth temperature below 0°C and a maximum growth temperature below 20°C. Thermophilic fungi have an optimum growth temperature range between 35 and 50°C, with a minimum of 20°C and a maximum of 50°C [9].

Reproduction in fungi can occur either sexually or asexually, and most often involves the production of spores. In many fungi, survival strategy involves two modes. As long as nutrients are available, the fungus is in the assimilative mode in which there is an emphasis on the accumulation of reserves of stored energy and the production of hyphae. The fungus switches to reproductive mode and produces spores if one or more of the following conditions are met: food runs out, reserves reach an appropriate level, staling factors build up or specific environmental signals are received [9]. Staling involves the accumulation of metabolites which slow or stop the growth of the organism which produces them. Spores range in size from 2μm to 100μm and may differ significantly in size, shape, method of formation and color. Formation of spores can occur from the fragmentation of undifferentiated hyphal elements or in sporocarps, which are fruiting bodies containing specialized hyphal branches. Asexual spores are divided into two groups, the sporangiospores are formed enclosed within a sporangium and the conidia are produced directly by the hyphae without any enclosing wall. Spore shape and method of formation are key elements in the identification and classification of fungi [7]. That fungi are found everywhere in the environment is a result of spore formation and dissemination. The ubiquity of spores ensures that when a nutrient source becomes available, fungi will be there to colonize it [9].

Fungi disseminate in the environment primarily through spore dispersal, but hyphal fragments may also be a means of dispersal. Most spores are designed for airborne dispersal although there are other methods such as dissemination by means of water [7]. In discussions of indoor air quality issues however, airborne dispersal is the primary concern. Release of spores into the air occurs either through passive or active discharge mechanisms [10]. Passive dispersion occurs through air movement, or the mechanical action of raindrops or animals. Active discharge mechanisms are common in fungi. Many spores are actively discharged by mechanisms that require moisture or high humidity which are used to produce high osmotic pressure and the bursting of an area of the fungal organism that results in the release of the spore [7].

Health Effects

Fungi can cause both allergic and infectious diseases. However, although most fungal spores are capable of causing allergic responses, very few are considered human pathogens [7]. Burge [7] states that possibly 85-90% of all mycoses are recurring infections of the skin or mucous membranes such as athletes foot or thrush. Examples of the relatively few fungi that are considered primary systemic human pathogens are *Histoplasma capsulatum, Blastomyces dermatitidis* and *Coccidiodes*. These fungi usually cause flu like symptoms that are most often not a serious health threat [8].

Persons who are immunocompromised are often infected by fungi that are not a health threat to non-immunocompromised individuals. Examples of these fungi are *A.fumigatus* and *Pneumocystis carinii* [8].

Exposure to fungi is most often associated with asthma, hay fever and hypersensitivity pneumonitis [7]. Nearly all fungi produce proteins or glycoproteins that can be highly allergenic and can cause hypersensitivity diseases in susceptible hosts. Between 10 and 60% of genetically susceptible persons develop immediate hypersensitivity (allergy) to fungi [8].

Hypersensitivity pneumonitis is a serious disease that can lead to permanent lung dysfunction. It is mediated at least partially by immunoreactants and results from intense repeated exposure to antigen-carrying particles that readily occur indoors [11]. The size of the particles is important in whether or not they will cause hypersensitivity pneumonitis. The particles must be small enough to reach the lower airways. These size particles are referred to as respirable. In general, particles smaller than 4 μm are considered respirable. There are many fungal spores that fit this criteria, and *Penicillium* species with 2 to 3 μm spores have been responsible for a number of hypersensitivity pneumonitis epidemics [11]. Other mold spores that are small enough to enter the lower airways are *Aspergillus, Candida, Geotrichium, Scedosporium, Paecilomyces* and *Scopulariopsis* species [12].

Mycotoxins are secondary metabolites of fungi that have no recognized role in the maintenance of fungal life [7]. Some of these mycotoxins are considered to be among the most carcinogenic of known substances. Toxigenic fungi are common contaminants of stored grain and other food products. One example of a toxin producing fungus is *Aspergillus flavus* which is a common contaminant of peanuts. This fungus produces aflatoxin, which causes liver damage at very low doses and is considered the most potent carcinogen known [9].

The overwhelming majority of mycotoxin research has focused on ingestion exposure [8]. It is commonly believed that more research on inhalation toxicity needs to be performed. Despite a number of reports linking inhalation of mycotoxins to human illness, there are very few well documented cases of inhalation induced mycotoxicosis [12]. Some fungi produce volatile organic compounds, which cause the musty odor associated with moldy areas, but it is unclear whether these pose any serious human health hazard [12].

Sampling

Burge [7] describes three types of sample collection methods for biological contaminants. These are observational sampling, bulk sampling, and air sampling.

Observational sampling includes a facility walkthrough in coordination with the use of the senses of sight and smell to determine whether there are any obvious sources of biological contaminant present. Bulk samples are portions of materials, such as wallboard or carpeting, that are tested to determine if fungal contamination is present [8].

When collecting fungal air samples indoors, outdoor samples should be collected simultaneously or in close temporal proximity with the indoor samples. These outdoor samples then serve as controls [12]. Relative indoor/outdoor comparisons of fungal concentrations and types are a primary basis for existing quantitative guidelines for fungi in indoor air. When indoor to outdoor comparisons are used, the absolute indoor concentration is evaluated as high or low only with respect to its level relative to the outdoor concentration. The other primary basis for these guidelines is baseline data [13]. When baseline data are used, indoor concentrations at a point in time in question are

compared to some value at a previous time when levels were known to be at "normal" levels. Various governmental agencies and private professional organizations have proposed guidelines for fungi in indoor air. Due to the lack of connection to human dose/response data, reliance on short term grab samples analyzed only by culture, and the absence of standardized protocols for measurement, there are no binding quantitative government regulations for bioaerosols in North America or the European Union. The Russian Federation is the only governmental agency that has binding quantitative regulations for bioaerosols [13]. In 1993, the State Committee for Hygiene and Epidemiological Surveillance of the Russian Federation revised the Maximum Allowable Concentrations of Harmful Substances to include airborne levels of fungi and bacteria. These regulations appear to be targeting the food processing and pharmaceutical industries. Concentration limits are set for individual species based on allergenicity in animal models and hazard class. The levels range from 10^3 cells/m^3 to 10^4 cells/m^3[13].

The California Occupational Safety and Health Administration recently amended its Code of Regulations, Title 8, Chapter 4(Division of Industrial Safety), Subchapter 7(General Industry Safety Orders), Group 2(Safe Practices and Personal Protection) Article 9(Sanitation), Section 3362(General Requirements) (a) to read:

> "To the extent that the nature of the work allows, workplaces, storerooms, personal service rooms and passageways shall be kept clean, orderly and in a sanitary condition. Building exteriors, interiors and environments that have a substance or condition that constitutes or contributes to a hazard covered by these orders shall be cleaned and maintained in such a manner that they will not give rise to harmful exposure, as defined in Section 5140. Visible mold or mold-infested building components such as ceiling tiles, wallboard, and carpeting inside a building are considered to be a type of unsanitary condition under this standard"

Section 5140 defines harmful exposure as "An exposure to dusts, fumes, mists, vapors, or gases: (a) In excess of any permissible limit prescribed by 5155; or (b) Of such a nature by inhalation as to result in, or have a probability to result in, injury, illness, disease, impairment, or loss of function"

This regulation does address mold, but not in terms of quantitative exposure limits.

There are a variety of methods of air sampling for fungi. As with other air sampling procedures sampling efficiency is of key importance to the process. The overall efficiency of a bioaerosol sampler can be divided into three components. These are inlet sampling efficiency, particle removal efficiency, and biological recovery efficiency [8]. The American Conference of Governmental Industrial Hygienists (ACGIH) [8] describes seven methods of particle collection commonly used by various bioaerosol air sampling devices: 1) inertial impaction, 2) multiple hole impaction, 3) slit sampling, 4) centrifugal sampling, 5) liquid impingement, 6) filtration and 7) gravitation or settling.

Impaction onto agar and spore trapping are the most common airborne fungal spore collection methods. Spore trapping is simply impaction onto an adhesive coated transparent surface such as a microscope slide [8].

Seltzer [12] states that air sampling for culture always underestimates the true bioaerosol concentration. This is the result of three things. First, some microbes grow poorly or not at all on standard media. Second, some microbes are non-viable at the time of sampling or are damaged by the sampling process. Third, the contaminant may be a non-viable product or portion of the microbe. Also for these reasons Seltzer [12] believes that sampling should include both culture (viable) and particle (non-viable) collection methods. Non-viable collection methods involve spore counting under a microscope.

Spore trapping, a non-viable sampling method in which spores are impacted onto on an adhesive surface, allows for accurate counting of total fungal spores, however, identification of all spores is often a problem because not all spores can be identified microscopically [8]. Gravity or settling plates significantly underestimate or fail to detect smaller biological contaminants, such as mold spores, that can remain airborne for long periods of time [12].

As a result of all the issues involved with air sampling, including a lack of governmental regulations and standardized protocols and the problems associated with culturing and identification, air sampling cannot satisfactorily be used to assess exposure to fungi in indoor air environments [14]. The research conducted in this project will be an important step towards the development of an effective method for the quantification of fungi in indoor air. Once a reliable method is developed, safe exposure levels can be established and quantitative regulations can be implemented.

Heating Ventilating and Air Conditioning Systems

Spores are transported from outdoor environments to indoor environments though windows, air intakes, cracks, occupants, etc. As a result, no interior environment is completely free of fungal spores, and in fact, many thousands or spores are present per gram of surface dust in most enclosed spaces [12].

The purpose of building heating ventilating and air conditioning (HVAC) systems is to mechanically ventilate the building and provide a comfortable environment for building occupants. Traditionally, the primary role of HVAC filtration materials was to protect HVAC equipment from dirt and dust that would eventually lead to expensive

equipment replacement and major maintenance. Today the additional roles of the air filtration system are to limit the contamination from microorganisms on the HVAC system equipment and to prevent contamination of the indoor air [15]. Although the role of the filtration system is to contain microbial contamination, there is the possibility that, under the appropriate conditions, the filters and other parts of the HVAC system may become sources of indoor microbial contamination. The HVAC system becomes part of a biological contamination problem by acting as a host, an incubator or propagator, or by transporting contaminants [1]. Because there will invariably be fungal fragments and spores entering the HVAC system, these fragments and spores will always be present on the system filter media. Viable fungi deposited in HVAC systems can multiply if adequate nutrients are available and temperature and humidity conditions are conducive to growth [16]. Because fungal fragments and spores are continually entering the HVAC system, the system filters can potentially be used as a record of fungal exposure. In order to use these filters as records of exposure, the environment in the HVAC system must be controlled in such a way that fungal particles collected on the filters do not have the opportunity to grow and colonize the filters.

Fungal Survival and Growth on HVAC Filters

In general, the filters of properly maintained heating ventilating and air conditioning systems are hostile environments for microbes. Fungal spores are quite resistant to the adverse environmental conditions found in most well maintained HVAC systems. While spore survival under these conditions is not uncommon, without sufficient nutrients and water, growth does not occur. The issue of whether or not fungi

can or do grow under "normal" HVAC operation conditions is very important to this study, since there would be a problem in extrapolating doses to building inhabitants from filter concentration if growth occurred on these filters.

A number of studies have examined fungal survival and growth on HVAC filters. In one such study Maus et al [17] studied the survival of mold spores in new and used air filter media (glass fiber, polyester fiber, and polypropylene fiber). HVAC filters were placed in an environmental chamber where relative humidity was controlled. These filters were then exposed to a known concentration of *A. niger* spores while a constant flow of clean air was drawn through them for 1 hour to 5 days. For new filter media, viability was not affected at relative humidity below 35%. At relative humidity of greater than 85% *A. niger* showed a slight decline in viability in a glass fiber medium and a more pronounced decline in polyester and polypropylene fiber.

The results for the used glass fiber medium were similar to the new glass fiber medium. However the decline in viability did not occur in the two other fibers.

The results from this study indicate that although relative humidity was high enough to support growth, there was no increase in viability on either the new or used filter media. This result is not surprising when considering the new filter media. In the case of the used filter media however, the filter cake most likely contained some material that could have served as fungal nutrients. The lack of growth under these conditions indicates that fungal colonization of HVAC filters is not always a definite occurrence, even under apparently ideal conditions.

Simmons et al[18] examined fungal colonization of three types of cellulosic HVAC filter media. Two of the filter media were treated with anti-microbial agents.

These agents were phosphated quaternary amine complex and silane-quaternary amine. The purpose of the study was to determine if the filter materials with the anti-fungal agents show a greater ability to inhibit fungal growth then materials without these agents.

Filter panels were placed onto the primary filter bank of a new industrial building and maintained for at least 40 days. At 7-day intervals up to 42 days, the filters were removed from the primary filter bank and cut into $4cm^2$ sections. Some sections were examined microscopically for fungi. The other sections were used as a source of innoculum for various agars. The agars were poured, at 46°C, through the sections of filters that lay in standard plastic petri dishes. The agar was poured so that the surface of the filter section was just covered. The plates were then incubated.

Culture yielded a slightly greater number of colonies on the untreated filters then for the treated filters. Although isolation of fungal fragments from the untreated filter media was common, there was no microscopic evidence of active colonization. The authors of this article concluded that while antimicrobial treatment of air filters may reduce the potential of fungal colonization and subsequent adverse health effects, untreated filter media in an ideally operated HVAC system will probably not be colonized by fungi within a 'normal' three month use life.

Kemp et al[16] examined the growth of microorganisms on filters loaded by having outdoor air flow through them. The experimental procedure involved the use of three 2ft x 2ft cross sectional ducts that ran horizontally through the test room. The ducts drew air from a window on one side of the room and exhausted it through a window on the other side of the room. Each duct was powered by an independent 2,000 cfm blower, which provided a face velocity of 500 fpm (2.5m/s) in the test ducts. The three ducts

each had three access doors for placement of test equipment in the duct. Over the course of 1 year, 3 types of filters (glass bag filter, polymer bag filter and an electronic air cleaner) were continually challenged with unconditioned and unfiltered 100% outdoor air. Surface samples of the filters showed that viable fungal spores were present; however no microbial growth was observed on any of the filters over the course of the year.

Martikainen et al[19] examined the occurrence of viable microbes on the HVAC filters of eleven pubic buildings. Three of the filters were glass fiber, one was impregnated (Viscosine®) glass fiber, and 7 were polyester fiber. The filters had been used for 6 to 24 weeks before involvement in the study. One gram of each filter material was eluted in 0.2 M buffered NaCl solution (PBS). These samples were then cut into pieces and homogenized with a mixer. This suspension was then plated on various agars and colonies were counted after incubation at 20°C for seven days. The counts of viable fungi were 7×10^2 to 2×10^5 colony-forming units per gram of dry filter material. These results indicate that not only do fungi survive on HVAC filters, but that their quantification is possible. The accuracy of this quantification cannot be determined, however relative levels could be used to compare different buildings and HVAC systems.

In this same study, Martikainen examined the environmental conditions (relative humidity and temperature) conducive to microbial activity on filters. The procedure used was to incubate pieces of the used filter materials at different relative humilities in 4 liter glass jars. Microbial activity was measured as CO_2 production in the jar. The different relative humidities examined were 75%, 82%, 95% and 98.5%. Two jars were incubated for each relative humidity, one at 5°C and the other at 20°C. The incubation period was 17 days. The researchers found that CO_2 was produced at all temperature and humidity

conditions tested, with the highest activity measured at 20°C and 95% relative humidity. They also found that activity depended more on humidity than on temperature. One of the study's conclusions was that microbial growth on HVAC filters is possible and could lead to the filter itself becoming a source of fugal contamination in the building.

Moritz, Schleibinger and Ruden,[20] investigated the survival time of outdoor microorganisms on air filters. The experimental procedure involved the use of a filter testing device called an Airotester (Camfil, Sweden). Using a ventilator, ambient air is drawn in and equally distributed to 4 test positions. Each test position contains a cylindrical support on which an air filter sample is placed. The velocity of air through each test position is 0.15 meters per second.

For purposes of the experiment the Airotester was operated on the roof (16 meters high) of a university building in Berlin, Germany. The filter samples used in the unit were from unused F7 (EU classification) fiberglass pocket filters. The experiment was run for sixteen days and filter samples were removed from the filter testing unit after different service lives and replaced with new samples. Test position 1 was replaced daily, position two was replaced every 2 days for the first 8 days of the experiment and every 4 days for the second 8 days of the experiment. Position 3 was replaced after 8 days. The position 4 filter was not removed until the completion of the 16 day period.

The procedure used to quantify the microbes on the filter was similar to that used by Martikainen. First, a 32cm^2 sample was cut out of the filter, then the sample was shaken in 50 ml NaCl solution with glass beads at a frequency of 150 cycles per minute for 60 minutes. The suspension was then plated and incubated for 4 days at 20°C before colonies were counted.

Survival time was determined by calculating the ratio of multiday samples to one day samples. The theory behind this calculation method was that if microbes were surviving on the filters for the entire duration of the study, then the multiday samples should produce higher colony forming units resulting in higher ratios. This was not what the results indicated. The ratios were generally quite low and the researchers concluded that mean survival time of microorganisms deposited on the air filters was 1 to 3 days.

The issue of fungal survival time on filters is a primary concern with respect to this study. If survival time is very short, then quantifying viable fungi and relating that quantification to an exposure period longer than that survival time is not possible. Moritz, Schleibinger and Ruden, conclude that survival time for all viable fungal particles is 1 to 3 days. By their own admission this result is contradicted by a number of other studies including those performed by Martikainen and Kemp which are described above. One possible explanation for their results is that the sampling took place during winter (November) 1993. Perhaps extended exposure to cold weather conditions adversely affected multiday samples in a way that was not a concern for one day samples. Another possible issue of concern is whether or not the Airotester results can be generalized to filters in an HVAC system. The conditions in the Airotester are not identical to those in a ventilation duct.

The results from the above experiments indicate that microbes do survive on HVAC filters. Most of the results indicate that microbial growth on these filters is not a serious problem. In the only study above to conclude that microbial growth on HVAC filters may be a problem, Martikainen based his conclusion on results from data obtained through the production of CO_2 in glass jars. There are two issues that arise from this

method. First is the fact that microbial CO_2 production in a glass jar is not a well established surrogate for growth in a ventilation system. Second, conditions in a glass jar are quite different from conditions in a ventilation system. For example, in ventilation system the organisms would be exposed high air velocities possibly leading to desiccation. Also, the experimental glass jars were maintained at constant temperature and relative humidity, conditions that would not likely exist in a filter bank.

Overall, the experiments described above indicate that it is not unreasonable to assume that fungi do survive on HVAC filters and that while growth on the filters of well maintained systems is unlikely, viable fungi on used HVAC filters can be quantified in the laboratory.

The following chapters present the findings of research in which filter quantification was assessed as a tool in viable indoor airborne fungal assessment. This research involved evaluation both in the field and under controlled laboratory conditions. This research progressed from the pilot field study presented in Chapter II to the laboratory validation presented in chapter III and finally to the larger scale field study presented in chapter IV. For the purposes of description in the following chapters, viable fungal concentrations are reported in units of colony forming units per cubic meter of air (CFU/m^3). In the laboratory study (Chapter III) the colony forming units refer specifically to fungal spores. In the field studies (Chapters II and IV), colony forming units refer to viable fungal particles in general, which include spores and hyphal (fungal body) fragments.

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CHAPTER II

VIABLE PARTICLE CONCENTRATION ON HVAC FILTERS AS AN INDICATOR OF RELATIVE VIABLE AIRBORNE PARTICLE LEVELS

Abstract

Airborne fungi and their spores are associated with asthma, hay fever and hypersensitivity pneumonitis. While it is known that indoor airborne fungal contamination causes adverse health effects in building occupants, there is no well-established, universally accepted method to accurately detect and quantify this contamination. This lack of a standardized method is a result of, among other factors, the high variation in fungal contamination over space and time, the problems involved in the collection of reliable long term-samples, and the difficulty in interpreting the results of sampling surveys. In addition to these three difficulties, the lack of standard detection and quantification methods impairs the ability of the scientific community to compare the results of different studies with uniformly collected data. This research was performed to evaluate a method of viable particle quantification of HVAC filters. The method involves the shaking of samples of filter materials in sterile saline followed by plating of the shaking solution.

The study involved two phases. The first involved the comparison of two HVAC filters from a building with a history of indoor air quality complaints in the space served by one of the filters. The space served by the second filter was separate from the first and did have a history of complaints associated with it. The second phase took place following remediation efforts in the same building. During this second phase the results obtained from the quantification of ten filters were compared with viable air sampling results in each of the areas served by the filters. Statistical analysis of the phase I results found a significantly greater number of viable particles on the complaint filter than on the non-complaint filter ($p<0.001$) . Phase II results did not uncover any statistically significant relationship between the results of air sampling and filter quantification results ($p=0.7218$).

Introduction

While indoor airborne fungal contamination is known to cause adverse health effects in building occupants, the lack of a well-established, universally accepted method to quantify this contamination has limited the ability of indoor air quality investigators to adequately evaluate airborne fungal levels in buildings. The lack of a standard detection and quantification method has impaired the ability of the scientific community to compare the results of different studies with uniformly collected data.

The purpose of this research was to evaluate the applicability of using results of viable particle quantification of HVAC filters as surrogate indicators of relative airborne particle levels. The relative levels compared in this research were between filters of units serving individual rooms in a typical academic university building. The first phase

of this two phase study involved the comparison of the results of a complaint with a non-complaint filter. The second involved the comparison of filter results with the results of viable air sampling in ten rooms each served by an individual filter. The filter quantification procedure used is described in detail in the following section.

HVAC systems are designed to introduce and circulate air throughout buildings. As air circulates, system filters remove contaminants to protect equipment and improve air quality. The quantification method used in this work takes advantage of this filtration by extracting viable fungal particles from these filters and using them to estimate the total number viable fungal particles in the filter. In order for this method to produce accurate results, two criteria must be met: 1) fungal particles collected on the filter must remain viable and 2) growth on the filters must not occur. The results of several studies have suggested that fungal particles remain viable and that growth is not a problem on filters of well maintained HVAC systems. [1,2,3,4,5]. Ultimately, this quantification method may be used to accurately estimate the average viable fungal concentration in the air of the occupied space served by the HVAC unit. In order for this estimate to be made from the filter quantification results, the flow rate through the filter and system runtime must be used to estimate the total volume of air through the filter. This volume could then be used as the denominator of a concentration with total viable particles in the numerator.

In this research small samples were removed from HVAC filters and the viable fungal load on these samples was quantified. The viable fungal load on the samples was used to extrapolate the fungal load on the entire filter. This total estimated filter fungal load was divided by an estimate of the total quantity of air flowing through the filter while it was in service. The resulting quantity was the viable fungal concentration of the filter

Materials and Method

Phase I: Comparison of HVAC Filters from a Complaint and a Non-Complaint Area of a Building

The viable fungal concentrations of two filters were compared to evaluate the hypothesis that the concentration on the complaint filter was significantly higher than the concentration on the non-complaint filter. The designation of the filters as either complaint or non-complaint was based on regular occupant indoor air quality complaints reported to the university health and safety office and administration prior to and during the entire time the test filters were in place. The designation was also based the presence or absence of minor visible mold growth on walls of the area served by the filter.

In this phase two separate filters serving two separate rooms on the second floor of an academic university building were examined and compared. The building from which the filters were removed is a six floor building housing administrative offices, faculty offices, and classrooms. The upper five floors of the structure are ventilated by individual units in each room. The majority are 1600 cfm closet units. Other rooms

contain smaller 900 CFM fan coil units. All fan coil and closet units are installed along the exterior wall of the building and receive their outdoor air directly from rectangular vents built into the exterior of the building. All individual room unit filters are Microsafe C&I synthetic panel filters (Koch Filter Corp., Louisville, KY). Filter size in fan coil units is 7.75" x 41.75" x 1", with an actual filter surface area exposed to airflow of approximately 206 in^2. Closet unit filter size in the room examined during this phase was 14" x 44.5" x 1", with an actual filter surface area exposed to airflow of approximately 486 in^2.

The complaint area is an academic classroom serviced by a closet unit, while the non-complaint room is administrative office space serviced by a smaller fan coil unit. Both filters were in service for the same time period from May through October 2002. A primary reason that this complaint room was chosen was that aside from visible mold on the walls, indoor air quality investigations conducted by the university environmental office had failed to discover any elevated levels (with respect to outdoor samples) of viable biological contaminants with viable impactor sampling.

Filter Quantification Method

The method used to quantify viable particles was adapted form Moritz and Martiny [6]. Filters were divided into four equally sized regions and one 4 in^2 sized square sample was cut from the center of each region using sterile scissors. Each filter sample was placed into a sterile 125 mL Erlenmeyer flask containing 50 mL of 0.9% sterile saline. The flasks were then shaken for either 30 or 60 minutes on an orbital shaker with shaking amplitude of 1" and a speed of 100 rpm. Upon completion of the

shaking cycle, 0.1 ml aliquots of each flask were plated in triplicate on both malt extract agar (MEA) and potato dextrose agar (PDA) plates (100 mm x 15 mm). The plates were then incubated at room temperature (21°C) for 96 hours at 75% relative humidity. Colonies were counted at the completion of the incubation period.

Phase II: Comparison of Single Stage Viable Impactor and HVAC Filter Fungal Quantification

This second phase was performed in the same building as phase I described above. During the time period between phase I above and the data collection described here, the building underwent efforts in an attempt to remediate any existing fungal problems. There were three aspects to these remediation efforts. First, each room was equipped with a small dehumidification unit. Second, the air intake grills for each of the ventilation units in the building were cleaned. The cleaning involved the removal of debris such as leaves, feathers, and other objects. Third, facilities maintenance personnel were instructed to regularly inspect all ventilation housing units and ceiling plenums and clean any suspected mold stains with Hillyard Q.T. ® Disinfectant Detergent.

This phase involved the analysis of data collected in ten separate rooms on the first floor of the examined building. All of the rooms evaluated were serviced by closet unit ventilation systems. Each of the systems had HVAC filters that were one of the following three sizes: 14" x 4 4.5"x 1", 16"x 44.5"x 1" or 12.5" x 44.5" x 1". All filters were in place over approximately the same 9 week period. Filters were installed between May 13-16 2003 and removed either July 24 or 25 2003. On July 30, 2003 two one minute and two two minute viable samples were collected onto malt extract agar with an

Andersen single stage viable impactor (Thermo Andersen, Smyrna, GA) in each of the ten rooms from which filters had been removed.

Filter Quantification Method

The malt extract agar plates on which the room air samples were collected were incubated at room temperature (21°C) for 96 hours at 75% relative humidity. Colonies were counted at the completion of the incubation period.

The HVAC filter quantification procedure used was similar to that described above in phase I. Filters were divided into nine equally sized regions and one 5 in^2 sample was cut from the center of each region using sterile scissors. Each filter sample was placed into a sterile 250 ml Erlenmeyer flask containing 100 ml of 0.9% sterile saline. The flasks were then shaken for 45 minutes on an orbital shaker with shaking amplitude of 1" and a speed of 130 rpm. Upon completion of the shaking cycle, 0.2 ml aliquots of each flask were plated in triplicate on potato dextrose agar plates (100 mm x 15 mm). The plates were then incubated at room temperature (21°C) for 96 hours at 75% relative humidity. Colonies were counted at the completion of the incubation period.

Results

Phase I

Analysis of variance (SAS v8.02, PROC GLM) of both media (PDA and MEA) data sets analyzed separately with complaint status as the independent variable indicated that a significantly higher concentration of viable particles (MEA $p<0.001$, PDA $p<0.001$) were recovered from the complaint filter (PDA 6.1 cfu/m^3; MEA 5.6 cfu/m^3) than from the non-complaint filter (PDA 1.6 cfu/m^3; MEA 1.7 cfu/m^3). Analysis of variance with shaking time as the independent variable did not indicate a statistically significant difference in concentration between 30 minute and 60 minute shaking time for either media (MEA $p=0.125$, PDA $p=0.904$).

Tables 2.1 and 2.2 summarize the results of CFU counts for PDA and MEA. The time column indicates whether the sample was shaken for 30 or 60 minutes. Colony forming units per cubic meter (CFU/m^3) represents an estimate of the average fungal concentration detected while the filter was in use. The following equation was used to calculate filter concentration in CFU/m^3:

Equation 2.1

$[(CFU/PV)*SV*FSA/SSA]/A_{total}$

where:

CFU=colonies per plate

PV=plating volume (0.2 ml)

SV=shaking volume (50 ml)

FSA=total filter surface area

SSA=filter simple surface area ($5in^2$)

and A_{total}=total airflow through the filter (m^3)

The design flow rates for the problem and non-problem units were 900 CFM and 1600 CFM respectively. Run time for both filters was approximately 259,200 minutes (180 days of 24 hour continuous operation). This run time corresponds to a total airflow of 2.3×10^8 ft^3 (6.6×10^6 m^3) for the non-problem fan coil filter, and 4.1×10^8 ft^3 ($11.7 \times 10^6 m^3$) for the problem closet unit filter.

Table 2.1 Summary of Quantification Results for Filter Samples Plated on Potato Dextrose Agar (PDA)

Complaint (Y/N)	Shaking Time (minutes)	[1]CFU	[2]CFU/m^3
Y	60	590.6	6.1
Y	60	622.3	6.4
Y	30	604.3	6.2
Y	30	547.3	5.7
N	60	183.3	1.4
N	60	189.6	1.5
N	30	236.6	1.8
N	30	227.6	1.8

1. Value indicated is the number of colony forming units counted on agar plate resulting from plating of 0.1 mL of shaking solution and is the average of three replicates.
2. Concentration extrapolated from CFU/plate, filter sample size, filter size, airflow rate, and unit run time Equation (equation 2.1, page 41): $[(CFU/PV)*SV*FSA/SSA]/A_{total}$
   Where CFU=colonies on plate, PV=plating volume (0.1mL), SV=shaking volume (50 mL), FSA=total filter surface area, SSA=filter sample surface area (2in^2), A_{total}=total airflow through filter (6.6 x 10^6 m3 for fan coil, 11.7 x 10^6 for closet unit)Example: [(590.6CFU/0.1mL)*50ml*486in^2/2in^2]/11.7 x 10^6 m^3 = 6.1 CFU/m^3

Table 2.2 Summary of Filter Quantification Results for Filter Samples Plated on Malt Extract Agar (MEA)

Complaint (Y/N)	Shaking Time (minutes)	[1]CFU	[2]CFU/m^3
Y	60	561.0	5.8
Y	60	503.3	5.2
Y	30	583.3	6.0
Y	30	519.0	5.4
N	60	134.3	1.0
N	60	204.0	1.6
N	30	262.6	2.0
N	30	282.6	2.2

1. Value indicated is the number of colony forming units counted on agar plate resulting from plating of 0.1 mL of shaking solution and is the average of three replicates.
2. Concentration extrapolated from CFU/plate, filter sample size, filter size, airflow rate, and unit run time Equation (equation 2.1, page 41): $[(CFU/PV)*SV*FSA/SSA]/A_{total}$
   Where CFU=colonies on plate, PV=plating volume (0.1mL), SV=shaking volume (50 mL), FSA=total filter surface area, SSA=filter sample surface area (2in^2), A_{total}=total airflow through filter (6.6 x 10^6 m3 for fan coil, 11.7 x 10^6 for closet unit)
   Example: [(561.6CFU/0.1mL)*50mL*486in^2/2in^2]/11.7 x 10^6 m^3 = 5.8 CFU/m^3

Phase II

The results of a regression performed with filter concentration as the dependent variable and two minute air sampling concentration as the independent variable indicated that statistically significant relationship did not exist between these two variables (SAS v8.02, PROC REG, p=0.963). Table 2.3 presents a summary of the results obtained from the performance and data analysis of phase II

Figure 2.1 is a graph of the two minute air sampling concentration value plotted against the calculated filter concentration for each room. Both concentrations are reported in units of CFU/m^3.

Table 2.3 Summary of Air Sampling and Filter Quantification Results

[1]Room	Air Sampling			Filter Quantification	
	[2]1 min CFU	[2]2 min CFU	[3]2 min concentration CFU/m^3	[4]Filter CFU	[5]Filter Concentration CFU/m^3
1*	5, 1	9, 9	158.9	35.7	0.44
2*	8, 6	8, 12	176.5	56.1	0.66
3*	2, 2	4, 0	35.3	58.9	0.69
4^	5, 5	6, 4	88.3	54.4	0.52
5^	1, 2	8, 6	123.6	48.5	0.47
6^	2, 2	7, 5	105.9	41.3	0.40
7^	2, 7	11, 13	211.8	36.7	0.37
8^	4, 5	8, 9	150.0	28	0.28
9`	1, 0	2, 4	53.0	29.3	0.26
10^	1, 8	4, 4	70.6	25.5	0.24

1. * ^ ` are filter size designations. * indicates 16" x 44.5" x 1", ^ indicates 14" x 44.5" x 1 " and ` indicates 12.5" x 44.5" x 1".
2. Comma separated values are the CFUs counted on each of the two malt extract agar plates used to collect air samples.
3. CFU/m3 calculated by averaging the two concentrations extrapolated from the 2 minute sampling CFU values
4. Value indicated is the number of colony forming units counted on agar plate resulting from plating of 0.2 mL of shaking solution and is the average of three replicates.
5. Concentration extrapolated from CFU/plate, filter sample size, filter size, airflow rate, and unit run time.
   Equation (equation 2.1, page 41): $[(CFU/PV)*SV*FSA/SSA]/A_{total}$
   Where CFU=colonies on plate, PV=plating volume (0.2mL), SV=shaking volume (100 mL), FSA=total filter surface area, SSA=filter sample surface area (5in^2), A_{total}=total airflow through filter.

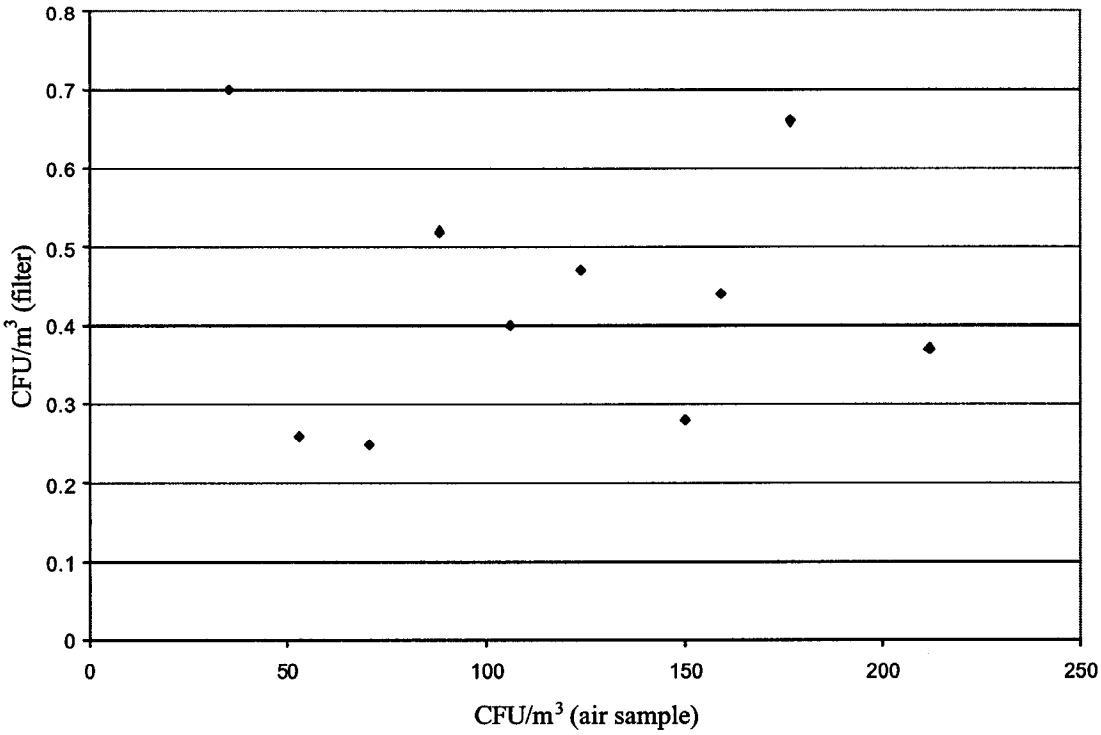

Figure 2.1 Air Filter Concentration vs Air Sampling Concentration Results

Discussion

Phase I, a comparison of filters from complaint and a non-complaint building areas yielded results indicating that a significantly higher concentration of viable particles were recovered from the complaint filter (PDA 6.1 cfu/m^3; MEA 5.6 cfu/m^3) than from the non-complaint filter (PDA 1.6 cfu/m^3; MEA 1.7 cfu/m^3) . These results suggest that the occupants of the complaint area were exposed to higher concentrations of fungal spores over the six-month period that the filters were in service. This potentially higher exposure, along with the fact that previous indoor air quality investigations uncovered no plausible explanation for the complaints, suggest that exposure to fungal spores may have played a role in triggering occupant symptoms. Previous indoor air quality investigations were conducted by members of the university health and safety office. The results of viable air sampling during these investigations indicated that the viable airborne fungal levels in the entire building, including the complaint area were sufficiently lower than outdoor levels to consider them elevated.

The results in Tables 2.1 and 2.2 represent paired data. The same shaking solution from each filter sample was plated onto both MEA and PDA. This was done to evaluate the consistency of results across both media. A two sample t-test indicated that there was no significant difference between the two media in the number of colony forming units recovered from each filter sample ($p=0.2581$). This consistency between the media strengthens the finding that the complaint filter did contain a significantly higher concentration of viable particles in and on it. The finding of no significant difference in particles recovered between the 30 and 60 minute shaking times was consistent with research previously conducted by Moritz and Martiny[6].

Although all of the filters examined in both phases of this research were identical in all aspects except for their length and width, the filter results obtained from the analysis of the data collected during phase II, are not directly comparable with those obtained from phase I. This is due to the fact that, 1) the filters were in place for and over different periods of time and, 2) the two HVAC systems evaluated in phase I were not examined in phase II. However, because all of the filters in both phases were of the same type and in service in the same building, a comparison of the estimated concentrations of viable particles collected in the two filter quantification procedures can be reasonably made. The results of this comparison suggest that remediation efforts that took place in the time period between the two phases were effective. The mean value of the filter concentration for all ten filters evaluated in phase II was 0.43 CFU/m^3. This value represents 11% of the average concentration (3.9 CFU/m^3) found when evaluating the PDA results of the two filters in phase I, and 7% of the average concentration (6.1 CFU/m3) found on only the PDA results from the problem filter. These comparisons show that the number of viable particles detected per unit volume of air handled on the post-remediation filters was substantially lower than that found on the pre-remediation filters.

In this study relative comparisons were made between rooms in one particular building. The quantification method and analysis of results described previously in this work can only be used to determine relative levels of viable airborne microorganisms. In order to use filters to estimate the actual average airborne concentration in the air that a filter has handled, the collection and removal efficiency of these organisms onto and off of the filter must be used in conjunction with HVAC system runtime and air flow. In its

current form, the quantification procedure can be used to compare separate areas of a building served by different filters. Another potential use of this method is as a baseline screening tool. A particular building may have its filters evaluated at a particular point in time when there is no suspected airborne viable particle problem and then re-evaluated at a later date when a problem is suspected. The initial evaluation could be at the completion of building construction, post re-mediation, or simply at another point in time when no problem is suspected.

Conclusions

Two conclusions can be derived from the filter quantification method. First, based on the results of the paired t-test that indicated plating on MEA and PDA yielded concentration results that were not significantly different it can be concluded that in future work either media could be used to perform filter quantification analysis and achieve similar results. This consistency of results across media also serves to strengthen the finding that there was a statistically significant difference between the complaint and the non-complaint filter. Second, based on the result that a statistically significant difference was not found between the 30 and 60 minute shaking times it can be concluded that the majority of viable fungal particles removed from the filter during the shaking procedure are drawn into suspension within the first 30 minutes. This finding is consistent with previous research [6] and as such provides evidence as to the applicability of the filter quantification method to this study.

The primary conclusion from this study is that the filter quantification method was sufficiently sensitive to distinguish between a complaint and a non-complaint filter.

A limitation of this portion of the research was that only two filters were evaluated. In the future, research of this type should involve the comparison of multiple complaint filters with multiple non-complaint filters in order to more accurately assess the differences between the two.

Analysis of Phase II results indicated the lack of a statistically significant relationship between filter and air sampling concentrations. This lack of a significant relationship, in combination with: 1) the fact that previous indoor air quality assessments did not find significantly elevated levels of contaminants and 2) the ability to distinguish between filters indicated in Phase I, suggests that the filter assessment method may work more effectively than traditional short term viable sampling methods in certain indoor environments. Two important aspects of this research that limit the ability to draw this type of conclusion are the durations of the impactor samples and the fact that the air sampling was performed after the filter loading period. The two minute impactor sampling time may not have been long enough in duration to collect a sufficiently representative sample that could be used to calculate an accurate room air concentration. That the air sampling was performed after the test filters were removed may not have allowed for the collection of samples that were comparable to the filter quantification results, especially if there were environmental conditions in the interim that caused significant changes in concentrations. Future research of this type should involve longer air sampling periods taken while the test filters are in service to avoid these limitations.

In order to drive the field of bioaerosol air sampling towards more uniform detection and quantification methods, new and innovative procedures must be developed and tested in an attempt to complement current sampling techniques. The development

of new techniques will, in time, lead to a reliable standard set of standard procedures for the assessment of bioaerosols during indoor air quality investigations. These standard procedures could then be performed in addition to other, more common, evaluation techniques used currently. These new procedures would serve two primary purposes. The first would be to serve as tools to better characterize fungal contamination. The second would be to serve as a means of standardizing data collection. The importance of having standardized data involves the ability to compare the results of different surveys. A standard procedure would allow for building evaluations conducted at separate times and in separate buildings to be comparable with one another. The work described in the this paper is meant to serve as an initial step in the evaluation of HVAC filters as potentially valuable sources of information in indoor air quality investigations involving the assessment of bioaerosols, and specifically viable fungal particles. Based on the results obtained from the research presented in this chapter, the work described in chapter three, in which filters were loaded with known concentrations of fungal spores in a controlled ventilation environment, was performed. The work in chapter three was intended to serve to characterize the viable particle collection and recovery efficiencies of test filters. This characterization is necessary if a filter quantification method is to be used as a means of determining the average viable particle concentration in the air that a filter has handled. The low filter concentration values found in this research (in comparison to air sampling concentrations) were most likely linked to the low collection efficiency of the examined filters (less than 20%) as well as to the fact that the filters were treated with an antimicrobial agent. Filters without an antimicrobial coating and of higher efficiency used in larger HVAC systems will likely be found to have much higher

CFU and concentration values when used in similar environments. If the collection and recovery efficiencies of the filters evaluated in this work were known, the average airborne concentration in the air that was handled over their service lives could be estimated base upon adjustment of the obtained results. Further work is ongoing to explore the possibilities of filter quantification as an indoor air quality assessment tool.

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CHAPTER III

DEVELOPMENT AND TESTING OF A METHOD TO QUANTIFY VIABLE FUNGAL PARTICLES ON HEATING VENTILATING AND AIR CONDITION SYSTEM FILTERS

Abstract

This research was conducted as a first step in the development of heating, ventilating and air conditioning filters as tools in the assessment of indoor airborne fungal contamination. Test filters were loaded with known quantities of fungal spores in a ventilation test chamber designed specifically for this reserach. The loading procedure involved aerosolization of fungal spore suspensions in aqueous 0.9% saline. The generation of fungal spore aerosol was accomplished through the use nebulizers designed for respiratory therapy use. The quantification method used to determine the fungal load on the filters involved the removal of small sample sections of the filter, the immersion of these samples in sterile saline, the shaking of the filter/saline combinations, and the subsequent plating of aliquots of the shaking solution onto potato dextrose agar. Initial results indicated fungal recovery of 0% to 5% of expected values from the filters. Through the implementation two sets of procedural and equipment modifications, fungal

recovery values were increased to as high as 93%. In some cases recovery values of greater than 100% were obtained. This was most likely due the breaking up of spore agglomerations during the loading and recovery processes.

Introduction

The purpose of this research was to evaluate the effectiveness of heating, ventilating and air conditioning (HVAC) filter examination as a means of quantifying the viable fungal load in the air of the occupied space served by that filter. The successful development of a method to utilize HVAC filters as a bioaerosol assessment tool would offer potential advantages over existing airborne fungal assessment and quantification methods. One advantage is related to the larger amount of air flowing through the filter as compared to traditional short term viable airborne sampling techniques. This larger quantity of air would allow for an improvement in the time integrated nature of the sampling. Another advantage is that reliance on expensive, cumbersome, and time consuming short-term air samples could be reduced.

During this research test filters were loaded with known quantities of fungal spores from one of two fungal species in a ventilation test chamber. The first species used was *Aspergillus niger.* This organsism was chosen due to its characteristic appearance on potato dextrose agar making it easy to identify. *A. niger* was used as the sole test organism in the development of the test procedure to prevent contamination problems from causing inaccuracies in the colony counting procedures used to quantify fungal load. Upon completion of method development with *A. niger*, *Penicillium chrysogenum* was added as a second test organism in the testing procedure.

The filter loading procedure involved the aerosolization of fungal spore suspensions of known concentrations in a ventilation test chamber. Aerosolization was accomplished through the use of nebulizers designed for inhalation therapy use. Following the loading procedure, fungal spores on the filters were quantified and these quantification results were compared with estimates of the number of spores originally loaded onto the filters. This comparison was reported in terms of filter efficiency (observed spores/expected spores) which was the outcome of interest. The purpose of this research was to determine the effectiveness of filter quantification as a means of estimating fungal spore load under controlled laboratory conditions. Degree of effectiveness was gauged in terms of filter efficiency, with higher efficiencies being associated with increased effectiveness. The effectiveness of nebulizers as generators of fungal spore aerosol was also evaluated.

Materials, Methods and Results

This work involved the initial development of the testing procedure and apparatus as well as two sets of modifications made to both the procedure and the apparatus to improve efficiency values. In the following sections a description of initial experimental configuration is followed by the modification details. Due to the progression of modifications to the methods involved in this research, results will be included after each stage of method modification.

Initial Procedure and Apparatus Configuration

Ventilation Test Chamber

Filter loading was performed in a test chamber consisting of a 16' length of 2' x 2' galvanized steel ducting, a test filter, two HEPA filters, and a fan, as shown in Figure 3.2. The design airflow in the duct was 1100 CFM. This air flow corresponds to an air velocity of 275 FPM which is a typical face velocity designed for HVAC filter banks. Appendix Tables A1 and A2 contain specific chamber air velocity data.

The HEPA filters at the entrance and exit portions of the test chamber were in place to assure that the air entering the chamber was free of any viable biological particles that might be present in the laboratory air, and that the air exiting the chamber was free of test organism spores.

Upstream of the test filter, a nebulizer was used to load the test filter with fungi by aerosolizing a fungal suspension into the duct airflow. The nebulizer was positioned in the duct in such manner that the emitted aerosol cloud was released upward and directly perpendicular to the air flow. Figure 3.1 displays a diagram of the nebulizer in the test chamber.

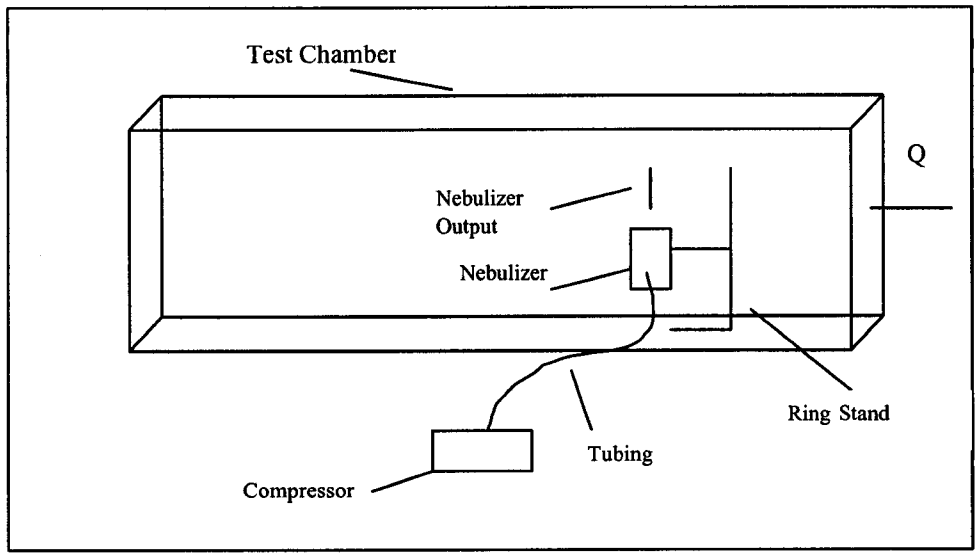

Figure 3.1 Nebulizer in Test Chamber

This nebulizer was operated by filling it with a liquid fungal suspension and subsequently using a compressed air source to cause the nebulizer to form aerosolize the liquid. Due to the relatively short distance between the nebulizer and the test filter, there was the concern that the aerosol released from the nebulizer may not become adequately mixed into the airflow. To address this issue, two sections of 1/8" mesh netting 6 inches apart were spread across an interior cross section of the duct perpendicular to direction of the air flow beginning 6 inches downstream of the nebulizer. The purpose of this netting was to improve the uniformity of the airflow through the duct system, leading to the more even distribution of fungal aerosol on the test filters.

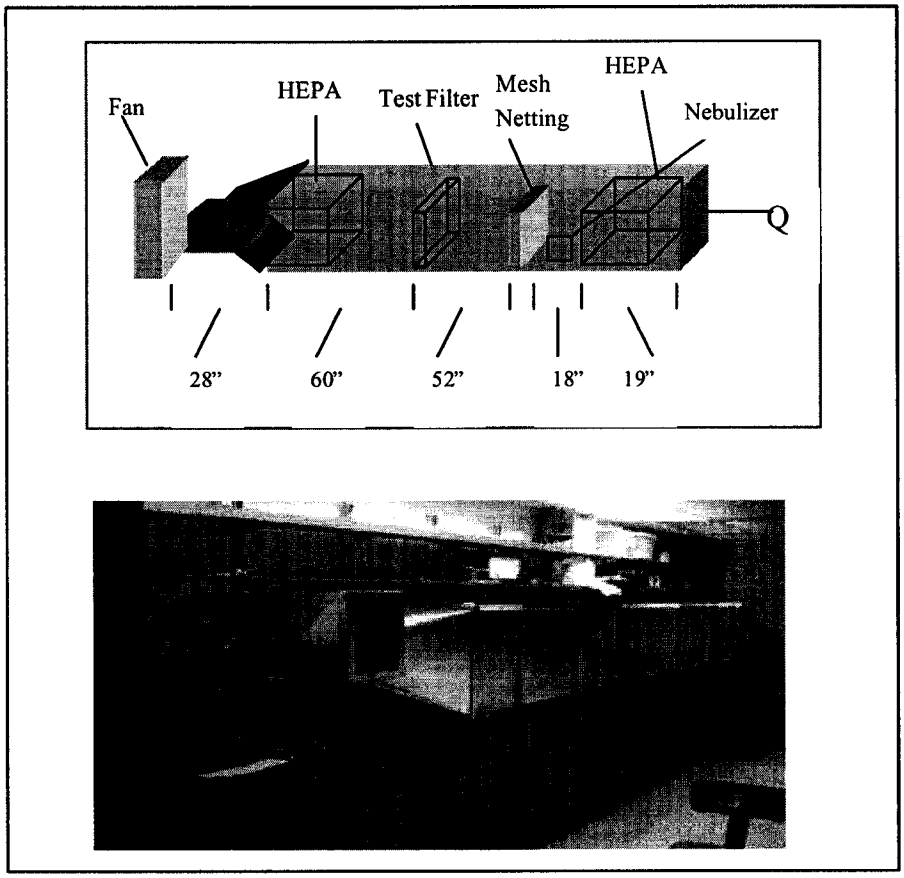

Figure 3.2 Ventilation Test Chamber Diagram and Photograph

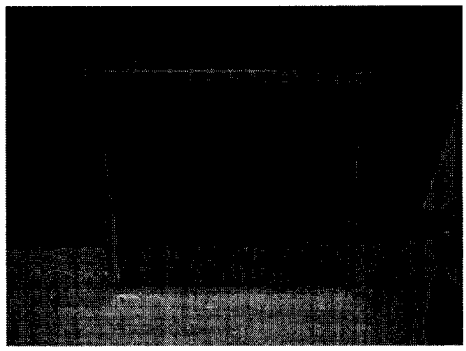

Figure 3.3 Mesh Netting to Improve Mixing

Generation of Fungal Suspension

The generation of the fungal spore suspension involved the growing of a lawn of the test organism on potato dextrose agar. The *A. niger* strain used was obtained from Raven Biological Laboratories (EZ-CFU™ *A. niger* ATCC# 16404, Omaha, Nebraska). Once the lawn was grown on the plate, the spores were harvested. Harvesting of spores involved four steps. The first was the flooding of the lawn-containing media plates with 0.9% sterile saline and the addition of sterile glass beads. The second involved the orbital shaking of the flooded plates for 15 minutes. Step three involved the transfer of the liquid from the flooded plate into a sterile test tube for vortexing. The final step consisted of the filtration of the vortexed suspension through two layers of Miracloth (Calbiochem, La Jolla, CA) to remove hyphal fragments. This suspension of harvested spores was nebulized to generate the fungal aerosol. The concentration of each spore suspension was determined through serial dilution and plating onto Potato Dextrose Agar (PDA, Difco, Kansas City, MO). The original spore suspension was serially diluted four times. Each of the four steps involved the dilution of 1 mL into 9 mL and the plating of 0.1 mL of the dilution in triplicate. The average concentration of the dilutions, as determined through plate counting and extrapolation, served as the estimate of the original suspension concentration.

Filter Loading

Prior to insertion into the chamber, test filters were placed into a filter frame and nine 5 in^2 sections of the filter were outlined with permanent marker. Figure 3.4 is a photograph of an outlined filter. The filter type used in this research was 3/4" Poly Media (pad filter) with Tack (viscous impingement coating) consisting of 75% fiber and 25% resin (Model VL-09PST, Filtration Group, Joliet, IL). For the purposes of descriptions in this work, this will be referred to as the pad filter.

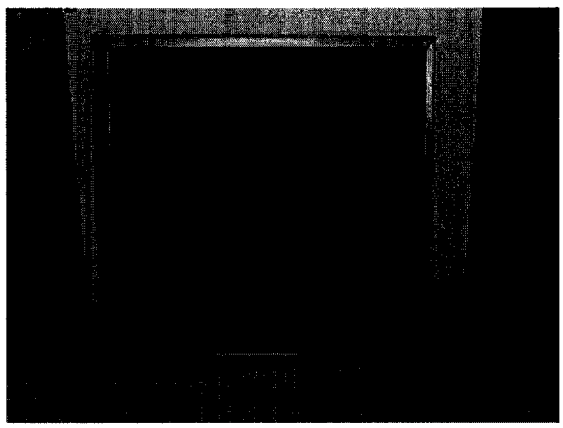

Figure 3.4 Test Filter with 5 in^2 Sections Outlined

Once the filter sections were outlined, the filter was inserted into the duct. Nebulizers were cleaned with 70% ethyl alcohol and allowed to dry before being filled with a small volume of the previously generated fungal spore suspension.

Two different nebulizers were used to generate the fungal aerosol. The volume of spore suspension used during each loading procedure was dependent on the recommended maximum fill volume for each of the devices. The first was the Devilbiss

4650D-621 (Sunrise Medical, Somerset, PA) which was filled with 6 mL of spore suspension during each loading procedure. The second was the Rinoflow® Nasal Wash and Sinus System (Respironics, Inc., Cedar Grove, NJ) which was filled with 8 mL of spore suspension during each loading procedure. Both nebulizers were operated at 15 psi. The Devilbiss nebulizer aerosolized the suspension at a rate of approximately 0.2 mL per minute. The Rinoflow nebulizer aerosolized the suspension at a rate of approximately 0.7 mL per minute.

The filled nebulizers were placed in the test chamber and held in place so that the aerosol outlet was directly in the center of the duct. Figures 3.5 and 3.6 are photographs of the nebulizers in the test duct.

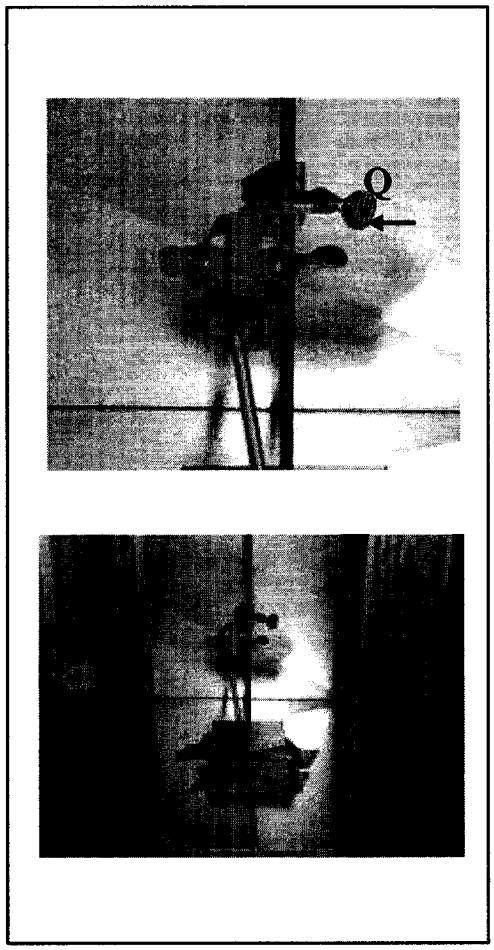

Figure 3.5 Devilbiss Nebulizer

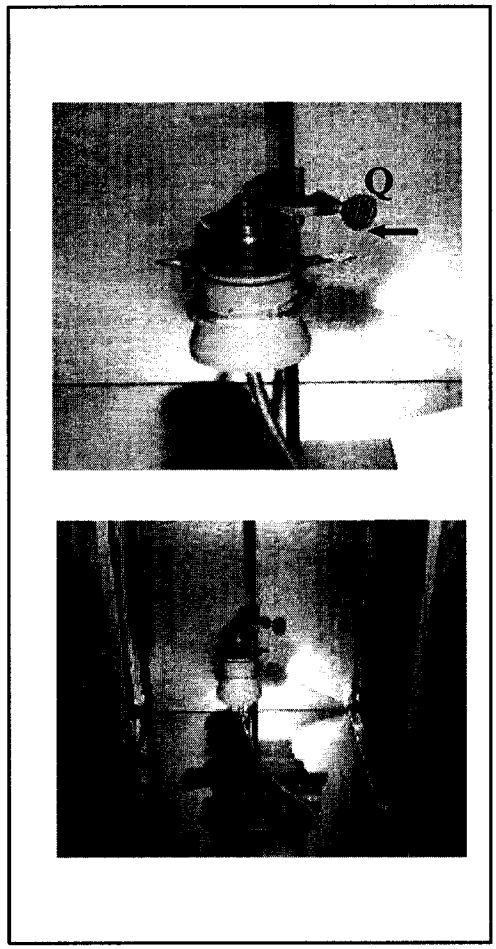

Figure 3.6 Rinoflow Nebulizer

Once the nebulizers were in place, the duct was sealed, the nebulizer pumps were turned on, and the flow of air through the duct was begun. The Devilbiss and Rinoflow nebulizers were run for 45 and 60 minutes respectively in order to allow for all of the liquid loaded into them to be aerosolized. At the completion of the nebulizer run period, the airflow was stopped and the filter was removed from the duct for quantification.

Filter Quantification

The method used to quantify viable particles was adapted form Moritz and Martiny [1]. Upon completion of a loading procedure, filters were removed from the test duct and each of the outlined 5 in^2 filter areas was cut out manually with sterile scissors. Each filter sample was then placed into a sterile 250 mL Erlenmeyer flask containing 100 mL of 0.9% sterile saline. Sterile saline was used instead of sterile purified water to be consistent with the method employed by Moritz and Martiny, from which this method was adapted. The flasks containing the filter/saline combination were shaken for 45 minutes on an orbital shaker with shaking amplitude of 1" and a speed of 140 rpm. Upon completion of the shaking cycle, 0.2 mL aliquots of each flask were plated in triplicate on potato dextrose agar plates (100 mm x 15 mm). The plates were then incubated at room temperature (21°C) for 96 hours at 75% relative humidity. Colonies were counted at the completion of the incubation period.

At the completion of each filter loading procedure, the nebulizer was removed from the duct and rinsed with a controlled volume (6mL or 8mL) of 0.9% sterile saline. The spore suspension resulting from this rinsing was then serially diluted and plated onto potato dextrose agar to determine its concentration. The total amount of fungal spores released from the nebulizer during each filter test was determined by subtracting the total number of spores remaining in the nebulizer after the procedure from the total number of spores estimated to be contained in the volume of suspension originally poured into the nebulizer. This net amount of spores released was assumed to be the amount loaded onto the filter. In calculating these spore loading values it was assumed that the number of

spores lost to duct surfaces would be insignificant in comparison to the total number of spores released from the nebulizer. As a result of this assumption, duct surface losses were not factored into the calculations.

The mean CFU count per plate resulting from the plating of the shaking solution for each 5 in^2 filter sample section was determined by taking the average value of the three replicates plated for each of the nine filter samples and then determining the mean of these nine CFU values. To obtain the HVAC filter recovery efficiency, this actual average spore count value per plate was compared to an expected number of spores that would be plated, based on extrapolating down from the net expected amount of spores loaded onto the entire filter, adjusted for: 1) the percentage of the entire filter represented by the 5 in2 section, 2) the dilution factors of the volume of the shaking solution (100 mL) and the volume plated (0.2 mL), 3) the 3.0 micrometer collection efficiency of the filter (in this case, 60%, obtained from the filter manufacturer), and 4) the extraction efficiency obtained experimentally by Moritz and Martiny (80%). The 3.0 micron collection efficiency was used due to the matching approximate size of *A. niger* spores. The following equation was used to calculate the expected number of plated:

Equation 3.1:

$[[[(S_L - S_n) / (F_{SA})] \times S_{SA}] / (V_S / V_P)] \times E_F \times K$

where:

S_L = spores loaded into nebulizer

S_n = spores remaining in nebulizer after loading period

F_{SA} = total filter surface area (484 in^2)

S_{SA} = filter sample surface area (5 in^2)

V_S = volume of shaking solution (100ml)

V_P = volume plated onto agar (0.2 ml)

E_f = 3.0 micron capture efficiency of filter (0.6)

K = shaking removal factor (0.8)

Initial Results

The loading and quantification procedure was performed a number of times with very poor results so they are not shown. Initial recovery efficiencies were in the range of 0 to 5 percent with no fungal spores found on many of the filter samples for both nebulizers.

Modification Procedure I

Two possible explanations for the poor results described previously were problems related to filter loading and filter extraction. The modifications described in this section address aspects of the filter loading step. Two critical aspects of the loading procedure that were dealt with in these modifications were the inefficient suspension and

aerosolization of the fungal spores and the inadequate mixing of the aerosol into the air stream. To address the suspension and aerosolization problem, 0.1% TWEEN-20, a surfactant, was added to the spore suspension after spore harvesting. The issue of inefficient mixing was addressed by placing obstacles in the path of the airflow downstream of the nebulizer. These obstacles were simply sheets of 6 mm plastic sheeting which acted as baffles. This plastic sheeting was secured over the two cross sections of 1/8" netting described previously. The center third of the upstream netting cross section was covered with this sheeting while the outer two thirds of the downstream sheeting were covered. Figures 3.7 and 3.8 are photographs of the covered upstream and downstream netting cross sections.

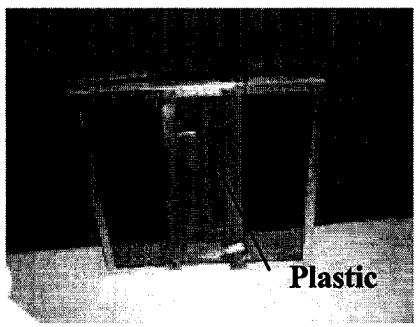

Figure 3.7 Covered Upstream Netting

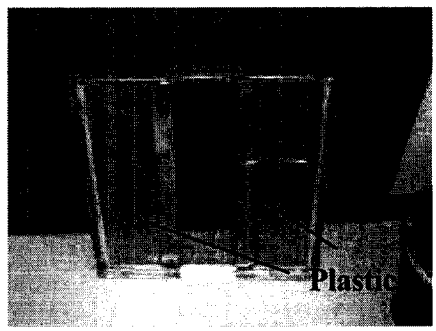

Figure 3.8 Covered Downstream Netting

Modification I Results

Loading procedures performed prior to the addition of the TWEEN-20 resulted in a visible spore film being left in the nebulzer following each loading procedure. Loading procedures performed after the addition of TWEEN-20 resulted in a lack of visible spore residue. Based on this lack of visible residue it was concluded that the

addition of TWEEN-20 improved aerosolization. The filter loading patterns resulting from procedures run following the addition of surfactant and prior to the covering of the mesh with plastic indicated that poor mixing was still a problem within the test chamber. Table 3.1 depicts a typical outcome demonstrating this pattern. Each of the 9 rectangular sections of this table corresponds to one of the quantified filter samples as displayed in Figure 3.4. The expected number CFUs per filter section sample for the loading procedure presented in Table 3.1 was 77.7. The average number of CFUs from all filter samples in this procedure was 11.2, resulting in a recovery efficiency of 14.4%.

Table 3.1 Growth Media Plate Quantification Results of 9 Filter Samples Loaded with the Rinoflow Nebulizer and the Addition of TWEEN-20

	Left	Middle	Right
Upper	0, 0, 0[1]	0, 0, 0	0, 0, 0
Center	1, 1, 0	97, 106, 85	0, 0, 0
Bottom	0, 0, 0	3, 4, 6	0, 0, 0

1. Each comma separated value in each box represents the number of CFUs (*A. niger* only) resulting from each of the replicates of plated filter sample shaking solution (0.2 ml plated) from that filter section..

Table 3.2 depicts the results of the first procedure performed with the plastic baffles in place. These results were typical of all procedures run in this configuration. This procedure was performed with the Rinoflow nebulizer. A comparison of the results displayed in Tables 3.1 and 3.2 indicates that the addition of the plastic sheeting baffles resulted in improved mixing of the fungal aerosol into the airstream when compared to results obtained prior to the addition of the baffles.

Table 3.2 Growth Media Plate Quantification Results of 9 Filter Samples Loaded with the Rinoflow Nebulizer, the Addition of TWEEN-20 and Plastic Sheeting (baffle) Covering Netting

	Left	Middle	Right
Upper	1, 1, 3[1]	1, 5, 2	3, 2, 3
Center	5, 4, 3	3, 4, 2	3, 3, 5
Bottom	1, 5, 8	5,13, 5	7, 4, 1

1. Each comma separated value in each box represents the number of CFUs (*A. niger* only) resulting from each of these replicates of plated filter sample shaking solution (0.2 ml plated) from that filter section.

The results presented in Table 3.2 demonstrate the improvement in fungal spore distribution over the surface of the test filter when compared to the results in presented in Table 3.1. However, there was no improvement in recovery efficiency over the results presented in Table 3.1. The expected number CFUs for the loading procedure presented in Table 3.2 was 35.4. The average number of CFUs from all filter samples in this procedure was 3.8, resulting in a recovery efficiency of 10.7%.

During this first modification procedure the effect that nebulizer distance from the first netting cross section had on recovery efficiency was evaluated to determine an optimal distance. Both nebulizers were used to load test filters at two distances in addition to the initial 6" distance. These distances were 1.5"(closest to the netting) and 12" (furthest from the netting). Table 3.3 summarizes the results from these loading procedures. An additional step was added to the loading procedure during these experimental runs. Following the initial loading period and with the test filter still in place, nebulizers were filled with either 6 mL or 8 mL of sterile saline and operated in the duct for a second period of either 45 or 60 minutes. At the completion of this time period this second step was repeated prior to the filter being removed and quantified.

The purpose of these steps was to aid in the suspension and aerosolization of as many spores as possible. This method resulted in there being no viable particles found in the nebulizer rinsate dilutions for either nebulizer. As a result in Table 3.3 the number of spores loaded into the nebulizer was equal to the number of spores released.

Table 3.3 Comparison of Filter Fungal Recovery Efficiency Based on Nebulizer Distance from Baffles

Nebulizer Distance from Sheeting	[1]Spores loaded into nebulizer	[2]Expected Average CFU Plate Value	[3]Experimental (observed) Average CFU Plate Value	% Recovery (Experimental/Expected)
Rino Flow Nebulizer				
1.5"	3.83×10^6	38.0	4.7	12.4%
6"	3.55×10^6	35.2	3.8	10.8%
12"	3.83×10^6	38.0	6.2	16.3%
Devilbiss Nebulizer				
1.5"	2.5×10^6	24.8	1.1	4.4%
6"	5.2×10^6	51.6	5.3	10.3%
12"	2.5×10^6	24.8	2.3	9.3%

1. Value obtained from the concentration of spores/ml in nebulized spore suspension (as determined through serial dilution) multiplied by the volume of suspension nebulized.
2. Value represents the average number of spores expected to be found on each plate inoculated with 0.1 ml of filter sample shaking solution. Calculated using the following equation (equation 3.1): $[[[(S_L - S_N) / (F_{SA})] \times S_{SA}] / (V_S/V_P)] \times E_F \times K$

   where S_L = spores loaded into nebulizer, S_n = spores remaining in nebulizer after loading period, F_{SA} = total filter surface area (484 in^2), S_{SA} = filter sample surface area (5 in^2), V_S = volume of shaking solution (100mL), V_P = volume plated onto agar (0.2 mL), E_f= 3.0 micron capture efficiency of filter (0.6), K = shaking removal factor (0.8)
3. Number of CFUs counted on PDA plates. Average of values for all 9 filter samples.

Modification Procedure II

Based on the results of the comparison of nebulizer types and distances from the upstream baffle, all of the procedures performed with modification procedure II involved loading the test filters with the Rinoflow nebulizer at a distance of 12" from the upstream baffle. The manufacturer reported mass median aerodynamic diameter for the Devilbiss nebulizer was 5 micrometers. The Rinoflow mass median aerodynamic diameter was reported to be within a range of 20 to 30 microns. During the design of these procedures it was assumed that the Rinoflow nebulizer would more effectively and efficiently suspend and aerosolize fungal spores than the Devilbiss nebulizer. The rationale behind this assumption was that the larger average aerosol particles generated by the Rinoflow nebulizer would more likely be of sufficient size to ensure a volume large enough to suspend the fungal spores. The results presented in Table 3.3 indicate that the highest efficiency resulted when the Rinoflow nebulizer was operated at 12" from the baffle. This outcome supports the larger aerosol particle rationale and thus was used as justification for the use of the Rinoflow for the remainder of the tests.

The modifications described in this section addressed aspects of the filter extraction step as well as additional improvements to filter loading. This second set of modifications included the addition of 0.1% TWEEN-20 to the filter shaking solution to aid in the removal of spores. TWEEN-20 (0.1%) was also added to the 0.9% saline harvesting fluid to further improve suspension and increase the number of spores collected during each harvesting procedure. Surfactant was not previously incorporated into the experimental procedure due to the fact that the method from which this procedure was adapted did not use it. Due to the fact the current work incorporated the 80%

recovery factor reported in the Moritz and Martiny work, the current method was maintained as closely to the method from which it was adapted as possible. A total of sixteen loading runs were performed with modification procedure II in which filters were loaded through the use of the nebulizer in the test chamber. Four of these filter loading runs involved the addition of TWEEN-20 to the shaking solution, but not the harvesting fluid. The other twelve procedures involved the addition of TWEEN-20 to both the harvesting fluid and the shaking solution. The harvesting fluid was the fluid used to flood the fungal lawn plates to collect the spores. The shaking solution was the solution in which test filter samples were shaken for the purposes of quantification.

In order to further explore whether the addition of TWEEN-20 to the shaking solution would significantly increase the removal of spores, two procedures were performed in which $5in^2$ filter samples had spore suspension added directly to their surface manually with a pipette. These two procedures were not performed in the test chamber and did not involve the use of a nebulizer. Filter samples were placed in sterile petri dishes and spiked with controlled volumes of spore suspension and shaken either with or without the addition of TWEEN-20 to the shaking solution. The first of these procedures involved the harvesting of spores without the addition of TWEEN-20 to the harvesting fluid. In this procedure the surfactant was added to the spore suspension immediately preceding suspension filtration. Loading of the filter samples involved manually dripping spore suspension directly onto the surface of the samples 0.1 mL at a time with a pipette. Two sets of five filter section samples each were spiked with amounts of spore suspension in increments of 0.1 mL from 0.1 mL to 0.5 mL. Upon completion of the loading procedure samples were allowed to dry for 24 hours prior to

being shaken for 45 minutes in 100 mL of 0.9% sterile saline shaking solution either with or without the addition of 0.1% TWEEN-20. A single unspiked control was run for each of the two shaking groups (TWEEN and NO TWEEN).

The second shaking procedure was similar to the first and differed only in two ways: surfactant was added to the harvesting fluid and the spiking was performed in triplicate for a total of thirty filter samples.

This set of modifications also included changes to the procedure to account for the number of spores lost to the baffles. This was determined in a manner similar to the filter quantification method. Squares of the same plastic sheeting material used to make the baffles were cut and cleaned on both sides with 70% ethanol before being taped to the baffle surface in a manner that left a square with an area of $5in^2$ exposed to the air flow. The upstream plastic baffle had three of these $5in^2$ pieces of plastic placed in the center of three equally spaced regions down its vertical center. Upon completion of a loading procedure, each 5 in^2 plastic square had its tape removed and was shaken for 10 minutes in either 50 mL or 100 mL of sterile saline before having 0.1 mL plated in triplicate onto potato dextrose agar. The CFUs counted on the plates were then used to extrapolate the total number of spores on the entire baffle surface. Figure 3.9 shows the plastic squares on the upstream baffle. Losses on surfaces other than the upstream baffle were not accounted for due to the results of a qualitative assessment of relative duct losses. During this assessment a filter loading procedure was performed with an unquantified spore suspension. This procedure was performed with 5 in^2 pieces of plastic taped at various locations along the four walls of the duct upstream of the filter as well as on the upstream and downstream plastic baffles. Quantification of these plastic sheeting pieces indicated

that the majority (>90%) of all quantified spores lost to duct surfaces were lost to the upstream baffle.

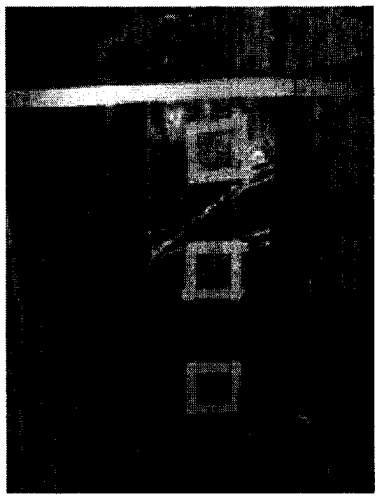

Figure 3.9 Plastic Squares Taped on Upstream Baffle

With these modifications in place a second organism and filter were evaluated in the testing chamber. The organism was *Penicillium chrysogenum* and the filter was a 24" x 24" x 2" Facet FME 40 Medium Efficiency Pleated Filter (Purolator Air Filtration, Henderson, NC) (70% 3 micron capture efficiency). For the purposes of description in this work, this will be referred to as the pleated filter. *P. chrysogenum* strain IBT 21424 was originally obtained from Dr. Kristian F. Nielsen (Technical University of Denmark, Department of Biotechnology, 221 Lyngby, DK-2800) by Dr. Chie Inumaru [(2)].

During these procedures each organism was loaded on each filter three times. The *P. chrysogenum* spore suspension generation process was identical to the *A. niger* suspension generation procedure. The pleated filter quantification procedure differed from the pad filter procedure in that five filter samples were shaken from each test filter.

A second difference was that 150 mL of shaking solution was used instead of 100 mL. Each sample was approximately 2.25" wide and three pleats in length. Figures 3.10 and 3.11 below depict a manually cut out filter sample and a filter with five samples removed.

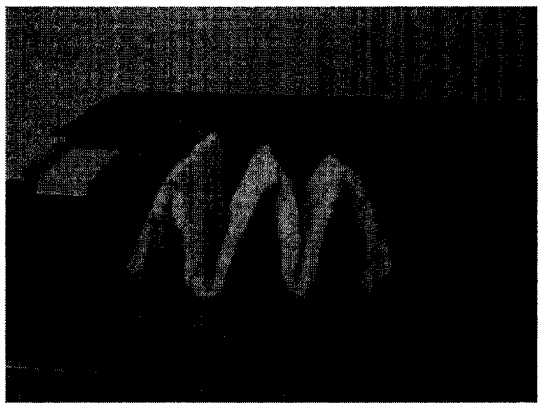

Figure 3.10 Pleated Filter Sample

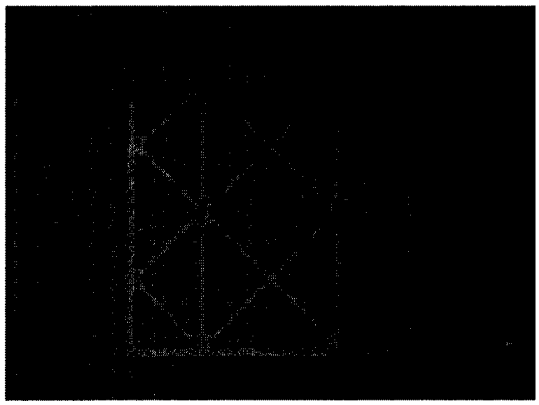

Figure 3.11 Pleated Filter with Samples Removed

Modification II Results

The four loading procedures in which TWEEN-20 was added to the shaking solution but not to the harvesting fluid resulted in an average recovery efficiency of 33.25%. All four of these loading procedures involved the loading of *A. niger* onto pad filters. A summary of these results is displayed in Table 3.4. The reported recovery efficiency value was calculated by dividing the actual (observed) colony forming units per agar plate by the expected value. The expected value was calculated using the following equation:

Equation 3.2:

$[[[(S_L - S_n - S_s) / (F_{SA})] \times S_{SA}] / (V_S / V_P)] \times E_F \times K$

where:

S_L = spores loaded into nebulizer

S_n = spores remaining in nebulizer after loading period

S_S=spores lost to plastic sheeting

F_{SA} = total filter surface area (484 in^2)

S_{SA} = filter sample surface area (5 in^2)

V_S = volume of shaking solution (100mL)

V_P = volume plated onto agar (0.2 mL)

E_f = 3.0 micron capture efficiency of filter (0.6)

K = shaking removal factor (0.8)

A paired t-test indicated that a significantly greater recovery percentage was acquired when manually loaded filter samples (spores harvested without TWEEN-20 in the harvesting fluid) were shaken in solution containing 0.1% TWEEN-20 than without the surfactant (SAS v8.02 PROC UNIV, p =0.027). This procedure involved a single replicate of each filter sample. A summary of this procedure is presented in Table 3.5. Analysis of variance analysis (SAS v8.02, PROC GLM), with TWEEN-20 (added or not added) as the independent variable, of the results from the manual loading procedure involving three replicates of each sample indicated a statistically significant difference in the recovery efficiency between manually loaded filter samples shaken with and without surfactant in the shaking solution ($p<0.0001$). In this procedure, TWEEN-20 was added

to the harvesting fluid. These results are summarized in Table 3.6. The procedures summarized in Tables 3.4 through 3.6 each involved the loading of only *A. niger* onto only pad filters.

Table 3.4 Fungal Recovery Efficiencies for the Four Filter Loading Procedures (*A. niger* onto pad filter) in Test Duct Adding 0.1% TWEEN to the Shaking Solution but not to the Harvesting Fluid.

Trial	[1]Spores Loaded into Nebulizer	[2]Spores Lost to Sheeting	[3]Spores Remaining in Nebulizer after Loading	[4]Expected Per Plate Average CFU Value	[5] Experimental (observed) Average CFU Per Plate Value	% Recovery (Experimental/ Expected)
1	5.20×10^6	2.05×10^6	3.6×10^5	27.6	11.8	42.8%
2	8.53×10^6	1.56×10^6	5.57×10^5	70.8	21.6	30.5%
3	16.11×10^6	1.83×10^6	1.65×10^5	128.4	52	40.5%
4	49.86×10^6	2.56×10^6	1.63×10^6	452.7	86.9	19.2%

1. Value obtained from the concentration (spores/ml) in nebulized spore suspension (as determined through serial dilution) multiplied by the volume of suspension nebulized.
2. Spores lost to sheeting calculated by extrapolating from the average CFU value resulting from shaking of $5in^2$ sheeting squares to entire plastic sheet. Each $5in^2$ sheeting square shaken in 50 ml or 100 ml 0.1% TWEEN sterile saline. The following equation was used to calculate this value: $(AV_P)(V_{SS}/V_{PL})(SA_{SHEET}/SA_{SAMPLE})$ where: AV_P=average number of CFUs resulting from plating of 0.1 mL of 5 in^2 plastic sheeting samples, V_{SS}=volume of shaking solution (50 or 100 ml), V_{PL}=volume of shaking solution plated (0.1 ml), SA_{SHEET}=surface area of entire plastic sheet (165 in^2), SA_{SAMPLE}=surface area of plastic sheeting samples (5 in^2).
3. Spores remaining in nebulizer determined through serial dilution of 8 ml of 0.9% sterile saline nebulizer rinsate.
4. Value represents the number of spores expected to be found on agar plate resulting from plating of shaking solution of each 5 in^2 filter sample. Calculated using the following equation (equation 3.2):$[[[(S_L - S_n - S_s) / (F_{SA})] \times S_{SA}] / (V_S / V_P)] \times E_F \times K$ where: S_L = spores loaded into nebulizer, S_n = spores remaining in nebulizer after loading period, S_S=spores lost to plastic sheeting, F_{SA} = total filter surface area (484 in^2), S_{SA} = filter sample surface area (5 in^2), V_S = volume of shaking solution (100ml), V_P = volume plated onto agar (0.2 ml), E_f= 3.0 micron capture efficiency of filter (0.6), K = shaking removal factor (0.8)
5. Number of CFUs counted on PDA plates. Average of values for all 9 filter samples.

Table 3.5 Manual Filter Loading Recovery Efficiencies (without TWEEN-20 in Harvesting Fluid)

Filter Sample	0.1% TWEEN Shaking Solution (Y/N)	Volume Loaded	[1]Expected CFU	[2]Experimental CFU	Experimental /Expected x 100%
1	Y	0.5 mL	116	55.7	48.0%
2	Y	0.4 mL	92.8	44.7	48.1%
3	Y	0.3 mL	69.6	55.3	79.4%
4	Y	0.2 mL	46.4	36	77.6%
5	Y	0.1 mL	23.2	19.6	84.4%
6 (control)	Y	0.0 mL	0	0	N/A
7	N	0.5 mL	116	43.3	37.3%
8	N	0.4 mL	92.8	22.3	24.0%
9	N	0.3 mL	69.6	22.7	32.6%
10	N	0.2 mL	46.4	11.7	25.2%
11	N	0.1 mL	23.2	16.6	71.6%
12 (control)	N	0.0 mL	0	0	N/A

1. Value represents the number of spores expected to be found on agar plate resulting from plating of shaking solution of each 5 in^2 filter sample. Calculated using the following equation: $(C_S)(V_L/V_{SS})(V_P)$

   where C_S=concentration of spore suspension (116,000 spores/mL), V_L=Volume of spore suspension loaded, V_{SS}=volume of shaking solution (100mL), V_P=volume of shaking solution plated (0.2ml)

2. Number of CFUs counted on PDA plates. Average of three replicates.

Table 3.6 Manual Filter Loading Recovery Efficiencies (TWEEN-20 in Harvesting Fluid)

Filter Samples	0.1% TWEEN Shaking Solution (Y/N)	Volume Loaded	[1]Expected CFU	[2]Experimental CFU	CFU Mean	Standard Deviation	[3]Recovery Efficiency
1-3	Y	0.5 mL	123.8	182 / 174.3 / 158	171.4	12.3	138.4%
4-6	Y	0.4 mL	99.0	139 / 144.6 / 137.3	140.3	3.8	141.7%
7-9	Y	0.3 mL	74.3	119 / 122 / 105.6	115.5	8.7	155.9%
10-12	Y	0.2 mL	49.5	76.6 / 73.6 / 66.6	72.3	5.1	146.0%
13-15	Y	0.1 mL	24.7	34.6 / 35.6 / 34.3	34.8	.68	140.9%
16-18	N	0.5 mL	123.8	85 / 64 / 49.3	66.1	17.4	53.9%
19-21	N	0.4 mL	99.0	78.3 / 60 / 58.3	65.5	11.1	66.2%
22-24	N	0.3 mL	74.3	42.6 / 54.3 / 46	47.6	6.0	64.2%
25-27	N	0.2 mL	49.5	12.6 / 28 / 23.6	21.4	7.9	43.3%
28-30	N	0.1 mL	24.7	11 / 11.6 / 12	11.5	.50	46.6%

1. Value represents the number of spores expected to be found on agar plate resulting from plating of shaking solution of each 5 in^2 filter sample. Calculated using the following equation: $(C_S)(V_L/V_{SS})(V_P)$ where C_S=concentration of spore suspension, V_L=Volume of spore suspension loaded, V_{SS}=volume of shaking solution (100mL), V_P=volume o f shaking solution plated (0.2mL)
2. Number of CFUs counted on PDA plates. Each value separated by "/" represents one filter sample and is the average of three replicates.
3. Experimental/Expected x 100% (recovery efficiency)calculated using average of three values from "Actual CFU" column.

There average recovery efficiencies for the twelve loading procedures in which *A. niger* and *P. chrysogenum* were loaded onto pad and pleated filters were as follows: 95.3% for *A. niger* on pad filters, 63.0% for *P. chrysogenum* loaded on pad filters, 275.5% for *A. niger* loaded onto pleated filters, and 111.0% for *P. chrysogenum* loaded onto pleated filters. The results from these procedures area summarized in Table 3.7. These were the only procedures involving the use of either *P. chrysogenum* or the pleated filters. Appendix Tables A3 through A13 contain the raw data from these procedures.

Table 3.7 Results of Trials Involving the Loading of *A. niger* and *P. chrysogenum* onto Pad and Pleated Filters Performed Under Modification II Conditions

Trial	Organism	Filter Type	[1]Spores Loaded into Nebulizer	[2]Spores Lost to Baffle	[3]Spores Remaining in Nebulizer After	[4]Expected Average CFU Recovery Value	[5]Actual Average CFU Recovery Value	Recovery Efficiency
1	*A. niger*	pad	26.57×10^6	2.57×10^6	1.52×10^6	223	135	60%
2	*A. niger*	pad	9.37×10^6	1.62×10^6	5.59×10^5	71	95	133%
3	*A. niger*	pad	5.72×10^6	8.59×10^5	1.08×10^6	37	35	93%
4	*P. chrysogenum*	pad	13.11×10^6	1.48×10^6	9.08×10^5	106	64	60%
5	*P. chrysogenum*	pad	7.40×10^6	2.79×10^6	3.75×10^5	42	22	53%
6	*P. chrysogenum*	pad	5.56×10^6	1.40×10^6	7.11×10^5	34	26	76%
7	*A. niger*	pleated	NR*	NR*	NR*	NR*	NR*	NR*
8	*A. niger*	pleated	6.44×10^6	1.04×10^6	2.32×10^5	66	111	168%
9	*A. niger*	pleated	1.91×10^6	8.04×10^5	1.60×10^5	12	46	383%
10	*P. chrysogenum*	pleated	12.61×10^6	1.73×10^6	8.51×10^5	127	96	76%
11	*P. chrysogenum*	pleated	6.31×10^6	8.43×10^5	3.02 x 105	67	38	57%
12	*P. chrysogenum*	pleated	5.5×10^6	2.05×10^6	5.00×10^5	38	76	200%

* Plates contaminated with multiple organisms. Accurate value not obtained.

1. Value obtained from the concentration (spores/ml) in nebulized spore suspension (as determined through serial dilution) multip lied by the volume of suspension nebulized.
2. Spores lost to sheeting calculated by extrapolating from the average CFU value resulting from shaking of $5in^2$ sheeting squares to entire plastic sheet. Each $5in^2$ sheeting square shaken in 50 mLor 100 mL 0.1% TW EEN sterile saline. The following equation was used to calculate this value: $(AV_P)(V_{SS}/V_{PL})(SA_{SHEET}/SA_{SAMPLE})$ where: AV_P=average number of CFUs resulting from plating of 0.1 mLof 5 in^2 plastic sheeting samples, V_{SS}=volume of shaking solution (50 or 100 mL), V_{PL}=volume of shaking solution plated (0.1 mL), SA_{SHEET}=surface area of entire plastic sheet (165 in^2), SA_{SAMPLE}=surface area of plastic sheeting samples (5 in^2).
3. Spores remaining in nebulizer determined through serial dilution of 8 ml of 0.9% sterile saline nebulizer rinsate.
4. Value represents the number of spores expected to be found on agar plate resulting from plating of shaking solution of each filter sample. Calculated using the following equation(equation3. 2, page 75):$[[[(S_L - S_n - S_s) / (F_{SA})] \times S_{SA}]] / (V_S / V_P)] \times E_F \times K$ where: S_L = spores loaded into nebulizer, S_n = spores remaining in nebulizer after loading period, S_S=spores lost to plastic sheeting, F_{SA} = total filter surface area (484 in^2 for pad filters and 1424.63 in^2 for pleated filters), S_{SA} = filter sample surface area (5 in^2 for pad filters and 24.19in^2 for pleated filters), V_S = volume of shaking solution (100mL for pad filter and 150mL for pleated filter), V_P = volume plated onto agar (0.2 ml), E_f= 3.0 micron capture efficiency of filter (0.6 for pad filter and 0.7 for pleated filter), K = shaking removal factor (0.8)
5. Number of CFUs counted on PDA plates. Average of values for all 9 filter samples.

Discussion

This work involved the development and validation of a procedure for the quantification of fungi on heating, ventilating and air conditioning system filters. Through modifications to the test procedure and apparatus, recovery efficiencies were improved from initial values of less than 5% to values of 60%, 93% and 133% for *A. niger* spores loaded onto pad filters. *P. chrysogenum* loading procedures on pad filters yielded efficiency results of 52%, 60% and 76%. The greater than 100% (133%) efficiency value obtained for one of the final *A. niger* loading procedures can likely be explained by spore agglomeration. When spores are initially harvested they agglomerate in suspension. Through the processes of nebulizing, and filter shaking, spore agglomerates break up. As a result, agglomerates that were counted as one CFU during the serial dilution and plating of the original spore suspension, may break up and become two or more CFUs in the filter sample shaking solution. The addition of TWEEN-20 to both the harvesting fluid and the shaking solution appear to have aided the suspension and breaking up of spores, however the surfactant does not act to break up all spore agglomerations. Figure 3.12 and 3.13 below depict spore agglomerates in a spore suspension following harvesting with 0.1% TWEEN harvesting fluid.

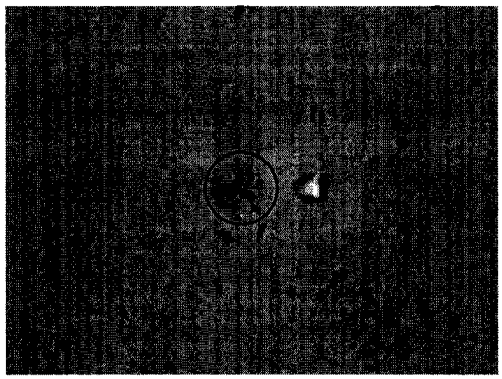

Figure 3.12 *A. niger* Spore Agglomeration

Figure 3.13 *A. niger* Spore Agglomeration with Spores Circled

Pleated filter loading results were more difficult to interpret than those for the pad filters due to both a wider range larger percentage recoveries. *P. chrysogenum* pleated filter efficiency results were 57%, 76% and 200%. The first two of these values are consistent with the pad filter results, however the 200% value is much higher. This increased efficiency can likely be explained by spore agglomeration. It is not unreasonable to expect that a poorly broken up spore suspension could yield an efficiency value two times an expected value. *A. niger* loading on pleated filters yielded efficiency values of 168% and 383%. No third value was reported due to contamination of the PDA

plates on which the filter shaking solution was plated. These efficiencies can also be reasonable explained by spore agglomeration. An examination of Table 3.6 demonstrates that the shaking procedure alone accounts for an expected average value of nearly 145%, which is the average recovery value (experimental/expected x 100%) for all samples in which TWEEN-20 was added to the shaking solution.. Due to vigorous nature of the nebulization process, adding the action of nebulization to shaking alone, could lead to a major difference in spore (colony) count between the suspension count and the final adjusted filter extraction count. In Figure 3.12 the circled mass in the center of the photograph is made up of approximately 15 spores. In Figure 3.13 there are 11 individual spores; however if the 6 spore and 2 spore chains remained intact upon plating onto agar, the counting procedure would determine there to be only 5 CFUs present. If the larger agglomeration in Figure 3.12 were to remain intact upon plating, the 15 individual spores could be counted as one CFU.

A comparison of the two filter types with respect to the recovery efficiency (Table 3.7) reveals that the pad filter values fell within a shorter range than the pleated filter values. The range of pad filter efficiencies was 53% to 133%, resulting in less than a factor of three difference between the lowest and the highest efficiencies. The range for the pleated filters was 57% to 383%, resulting in nearly a factor of seven difference between the lowest and the highest efficiencies. This difference recovery efficiency indicates that certain filter types may be better suited for use in the quantification procedure.

Variations in quantification accuracy may also be associated with factors other than spore agglomeration. One of these factors is spore loss to duct and nebulizer

surfaces. The limited space in which to build the duct did not allow for sufficient length to allow for the generated aerosol to mix fully into the airflow before being captured by the test filter. While the inner galvanized steel surfaces of the duct were not significant sources of fungal spore loss, the baffles designed into the duct system to address this issue were a source of loss in the loading process and any error in baffle quantification loss would lead to an error in overall process efficiency. It was assumed that a significant number of the aerosolized spores would impact on the baffle surfaces and that the actual number of spores lost would be proportional to the number of spores in the aerosolized suspension. However, an examination of Tables 3.4 and 3.7 indicates that estimated spore loss to baffles was held within a range of approximately 0.8 to 2.8 million spores. This range is relatively narrow when compared to the range of total spores loaded into the nebulizer, which was 1.9 to 49.9 million. This narrow range of baffle loss may indicate that there are a maximum number of spores that can be accurately quantified using the 5 in^2 plastic sample quantification method and that above this maximum spore count value an accurate spore quantification value cannot be obtained. It may also be the case however, that the quantification procedure was accurate and that the number of spores lost to the baffles was not directly related to the number of spores in the nebulized suspension.

The manual filter loading procedure results indicate that the addition of TWEEN-20 to the filter shaking solution results in an increased spore recovery efficiency. A paired t-test performed on the data presented in Table 3.5 indicated that a significantly greater recovery percentage was acquired when filter samples were shaken in solution containing 0.1% TWEEN-20 as opposed to samples shaken without TWEEN-20 in the

shaking solution. This is in agreement with the analysis of the results in Table 3.6. Further examination of Table 3.5 reveals a trend in which recovery efficiency appears to increase with decreasing filter sample spore load. This trend is particularly evident for the group of samples in which TWEEN-20 was added to the shaking solution. The NO TWEEN group appears to have a sudden increase in recovery efficiency from filter samples with 0.2 mL to 0.5 mL (7, 8, 9, 10) to the one with 0.1 mL on it (11). This trend may be an indication that quantification is more accurate at lower filter loads. The results in Table 3.6 also indicate a trend in which the variability in plate CFU counts within the three replicates decreases as the number of spores loaded onto the filter decreases. This may also be an indication that quantification is more accurate at lower filter loads. This pattern may prove valuable when this filter quantification method is evaluated in field studies.

That the addition of TWEEN-20 during any stage of the filter loading and quantification process serves to increase spore recovery efficiency is supported by the increase in recovery efficiency seen in the filter samples shaken with TWEEN-20 in Tables 3.5 and 3.6. Whether this increase in efficiency is due to increased spore extraction from filters, increased breakup of spore agglomeration, or some other reason cannot be determined by these results, however the outcomes of this research indicate that the addition of the surfactant serves to improve the processes of harvesting, suspension, nebulization and extraction.

The variation in filter extraction efficiency was not the only concern with respect to the filter loading procedure. ANOVA analysis of the data summarized in Appendix Table A14 indicated that the middle and bottom sections of the pad filter yielded

significantly higher recovery percentages than did the top section (SAS v8.02 PROC GLM, $p<.0001$). The same is true of the pleated filter data summarized in Table A14 (SAS v8.02 PROC GLM, $p<0.0001$). When the data was used to evaluate the pad filter in vertical sections, there was no significant difference found between the left, center, and right sections of the filter (SAS v8.02 PROC GLM, $p=0.0994$). This vertical analysis did however determine there to be a statistically significant difference in recovery percentage between the left and center sections of the pleated filter (SAS v8.02 PROC GLM, $p=0.0099$). This variation in recovery efficiency is due primarily to the lack of adequate mixing in the duct between the nebulizer and the test filter. This inadequate mixing results from the short distance between the nebulizer and filter. While under ideal conditions spore loading would result in a more even distribution across the test filters, this uneven distribution is not a major concern due to the fact that filters in building ventilation systems are often loaded unevenly.

Conclusions

The primary conclusion drawn from this work is that quantification of viable spores from HVAC filters is possible and produces results indicating that the method merits further scientific evaluation. A second conclusion is that nebulizers can be effective tools in the generation of fungal spore aerosol used in bioaerosol research. The development of nebulizers as tools in viable particle research would provide an inexpensive and easy to operate option for researchers wishing to generate fugal spore aerosols.

Two limitations of this work are that a limited number of organisms and filters were evaluated. Both organisms involved in this research were in the 3.0 micrometer size range. In typical occupied indoor environments, there are numerous species of organisms of a wide size range present. In order to more accurately assess the usefulness of this procedure in the field, laboratory testing involving a wider range of organisms and filter types should be performed. A third limitation is that the actual size range of fungal spores within an individual species varies. In this research the manufacturer reported 3.0 micrometer collection efficiencies of the filters were used in all expected plate spore count calculations, when in actuality the spore collection efficiency of each of the two filters may have varied from this value.

Another limitation of this work is the lack of accurate loss quantification. In order to accurately calculate recovery efficiencies, the degree of spore loss to the nebulizer and duct surfaces must be well characterized.

While the above mentioned limitations did exist, the combination of the results obtained in this research and in chapter two justify further filter quantification work in the field. The next logical step in the development of HVAC filters as airborne fungal assessment tools is to perform field studies in which building system filters undergo quantification. Chapter four describes the procedures and results of such a study. The work presented in chapter four involved the comparison of filter quantification results with average viable airborne sampling results taken while the filters were in service. In this study viable particle counts obtained from filters were normalized by the amount of air flowing through the filters during their service lives. These normalized values were then reported as detected concentrations.

References

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CHAPTER IV

COMPARISON OF HVAC FILTER QUANTIFICATION AND VIABLE AIR SAMPLING AS AIRBORNE FUNGAL ASSESSMENT TOOLS

Abstract

The purpose of this research was to assess viable particle quantification from building HVAC filters as a means of assessing indoor airborne fungal levels. This assessment was performed through the comparison of filter quantification results to the results of single stage viable impactor samples taken at regular intervals while test filters were in service. Research was conducted in three homes, an administrative office building, an academic research facility and an outpatient healthcare facility. Test filters were installed in ventilation systems for periods ranging from 4 days to 7 weeks (loading periods). Results of data collected in the outpatient facility and three homes indicated statistically significant relationships between filter quantification and air sampling results over short term (1 week and 2 week) loading periods, but not over a longer (7 week) term loading period. The results of longer (6 week) loading periods in the research and administrative facilities did indicate the presence of a statistically significant relationship. Results also indicated that the filter quantification procedure, with filters loaded for one week, demonstrated an increased ability to distinguish between different building air concentrations when compared to single stage impactor sampling.

Introduction

The results of short term indoor viable fungal air sampling are often very difficult to interpret. The constant fluctuation of airborne concentrations over space and time is a primary factor contributing to this difficulty. The development of sampling techniques which allow for reliable long term viable sampling would provide the potential for results that, in combination with traditional sampling results, could more accurately characterize the airborne fungal load of indoor environments. In this research the filter quantification procedure described in the previous chapters was used to quantify viable fungal particles on filters removed from building HVAC systems. The results of filter quantification were compared with traditional air sampling results taken while the filters were in service.

Materials and Methods

The quantification of viable fungal particles from HVAC building filters was examined as a means evaluating the relative levels of viable airborne fungal particles in six buildings. The buildings consisted of a single story outpatient health care facility, a four story administrative building, a four story academic research facility, and three residential single family homes. Filters were installed and allowed to remain in the HVAC system of each building for periods of time ranging from four days to seven weeks. While the filters were in place, Andersen single stage viable impactors (Thermo Andersen, Smyrna, GA) were used to collect air samples onto malt extract agar (MEA) at 28.3 liters per minute within areas of the buildings served by the HVAC system. Concentrations were determined in CFU/m3 after adjusting the CFU/plate values using

the Positive-Hole Correction procedure which takes into account the possibility of collecting multiple particles through a hole [1].

In order to have an indication of the variability of the spore distribution across the filter surface, the coefficient of variation for each filter was calculated by dividing the standard deviation of the filter section values in CFU/m^3 by the mean of those values. The experimental filter quantification value for each filter sample was calculated using equation 2.1 (Chapter II, page 31) in which the colony forming units per plate were used to extrapolate the number of viable particles on the entire filter surface, which was then divided by the total airflow through the filter to estimate concentration.

Research Buidings

The six buildings involved in this research were divided into two groups, based on the type of ventilation system contained within the building. The purpose of this division was to separate the buildings into groups with similar HVAC systems air volumes, filters, and general design. The first group consisted of the outpatient health care facility and the three residential dwellings. The second consisted of the administrative office building and the research facility.

Outpatient Health Care Facility

The outpatient health care facility is served by a decentralized HVAC system with thirteen zones each served by ten individual HVAC residential style air handling units. All units ran continuously 24 hours per day. This research involved the evaluation of five of the thirteen unit filters. Units and the corresponding areas that they served were

designated a number 1 through 5. Impactor sampling was performed in one location representative of the entire area served by each of the air handling units. Area 1 is a nursing triage area with a history of regular indoor air quality complaints associated with it. This area also had a history of ongoing roof leaks resulting in moisture being absorbed into the carpet. In addition, Area 1 also served as a storage location for medical records. Area 2 is a waiting room with no history of regular roof leaks or indoor air quality complaints. Area 3 is also a waiting room with no history of roof leaks within the previous year, but with a history of infrequent indoor air quality complaints. Area 4 is an x-ray waiting area and nursing station that had recently been remodeled and did not have a history of leaks or complaints since the remodeling. Area 5 is a waiting area and had a history of ongoing roof leaks that did not result in moisture being taken up by the carpet. This area also had a history of infrequent indoor air quality complaints.

Units 1, 3, 4 and 5 all were served by 16" x 25" x 1" pleated Facet FME 40 Medium Efficiency Pleated Filters (Purolator Air Filtration, Henderson, NC). Unit 2 was served by a 20" x 25" x 1" filter of the same type.

Table 4.1 presents a summary comparison for the five areas including flow rate and face velocity of the ventilating unit as well as occupant complaint frequency and roof leak status. Velocity measurements were taken with an Alnor Thermoanemometer model 9870 (TSI, Inc., Shoreview, MN) in the return and outdoor supply ducts to calculate flow rates and outdoor air percentages. For the purposes of airflow calculations, an effective filter surface area exposed to airflow of 322 in^2 (14" x 23) was measured for the 16" x 25" x 1" filters. An effective area of 414 in^2 (18" x 23") was measured for the 20" x 25" x 1" filter.

Table 4.1 Comparison of Outpatient Facility Air Handling Units and Areas

Unit	Airflow (CFM)	Face Velocity (FPM)	IAQ Complaints	Ongoing Roof Leaks
1	686	307	regularly	yes
2	1935	673	no	no
3	1343	601	infrequently	no
4	878	393	no	no
5	1005	449	infrequently	yes

Sampling activities began in this facility on February 4, 2004 and continued until April 15, 2004. During this time period sampling was conducted in each of the previously described areas of the facility as well as outside of the building on seventeen (approximately evenly spaced) separate occasions. On each occasion, two side by side samples were taken onto MEA plates with Andersen single stage viable impactors in each study area. Table 4.4 details the number of times air sampling was performed during each filter loading period. The sampling durations of the samples collected during the first three sampling occasions were 5, 5 and 8 minutes. The remaining indoor samples were collected for 15 minutes. The final six outdoor samples were collected for 10 minutes.

During the data collection period three test filters were loaded and quantified in each air handling unit. Used filters were removed and new filters installed on February 4, March 26, April 9 and April 16. This filter change out schedule resulted in three test filters being loaded for approximately 7 weeks (52 days), 2 weeks (15 days) and 1 week (8 days). The total airflow through each filter for each loading period is presented in Table 4.2.

Table 4.2 Total Air Volume (cubic meters) Through Outpatient Facility Filters for All Loading Periods

	1 Week Loading Period Total Air Volume (m^3)	2 Week Loading Period Total Air Volume (m^3)	7 Week Loading Period Total Air Volume (m^3)
Unit 1	2.24×10^5	4.20×10^5	1.46×10^6
Unit 2	6.31×10^5	1.18×10^6	4.10×10^6
Unit 3	4.38×10^5	8.22×10^5	2.85×10^6
Unit 4	2.87×10^5	5.37×10^5	1.86×10^6
Unit 5	3.28×10^5	6.16×10^5	2.13×10^6

Filter quantification was similar to the method described in the previous chapters. Using a template, each filter had either six or seven 5 in^2 sections cut out manually and placed in either 50 ml or 75 ml of 0.9 % sterile saline with 0.1% TWEEN-20 in 250 ml Erlenmeyer flasks. The flasks were then shaken for 45 minutes on an orbital shaker with shaking amplitude of 1" and a speed of 145 rpm. Upon completion of the shaking cycle, 0.1 ml aliquots of each flask were plated in triplicate on potato dextrose agar (PDA) plates (100 mm x 15 mm). The plates were then incubated at room temperature (21°C) for 96 hours at 75% relative humidity. Colonies were counted at the completion of the incubation period. The five areas in this facility were ranked with respect to the relative airborne fungal concentration expected to be discovered in each area. This ranking was based on occupant complaint frequency, the presence and severity of roof leaks or other water damage, and any other relevant factors presented by the facility engineer (Table 4.1). Due to regular roof leaks, a history of minor water damage, and frequent occupant complaints, area 1 was expected to have the highest airborne concentration of the five areas. Based on infrequent complaints and the presence of roof leaks, area 5 was expected to have the second highest airborne fungal concentration. Area 3 was expected to have the third highest airborne concentrations based on the fact that while roof leaks were not present, there were infrequent occupant complaints. Areas 2 and 4 were

expected to have the lowest airborne concentrations based on a lack of occupant complaints, the fact that roof leaks were not present, and a lack of any water damaged areas. Of these last two areas area 4 was expected to have the lowest airborne concentration based on the fact that the area had been recently remodeled. The new building materials and upholstery in this area were less likely to be reservoirs of fungal particles than the materials and upholstery in other areas. Based on the information described above the areas were ranked in order of expected viable airborne fungal concentration as 1, 5, 2, 3, 4.

Residential Dwellings

The three homes involved in this research were each located in the same small Midwestern American city and are single family dwellings with basements and ducted ventilation systems. The HVAC unit in each of these homes is of the same type as those found in the outpatient facility. The systems in both the homes and the outpatient facility were single filter, low volume systems with little to no outside air and similar volumes of air handled. During the data collection period (while test filters were loaded and air samples were being taken), each of the home units was operated continuously (24 hours/day). Home 2 had no history of owner indoor air quality concerns, while the owners of home 1 had minor concerns of potentially elevated mold levels in the basement and the owners of home 3 had serious concerns related to mold odors and respiratory symptoms potentially related to airborne allergens. Home 1 had a semi-finished basement (carpeting with cinder block walls), home 2 had a finished basement and home 3 had an unfinished, partial dirt floor basement with a history of elevated moisture levels.

The owners of homes 1 and 3 were each dog owners. There were no pets in home 2. Table 4.3 provides a summary of this information. The filter loading and air sampling period in each of these dwellings ran from May 25, 2004 to May 28, 2004. One filter was loaded in each dwelling over this time period. Table 4.4 details the number of times air sampling was performed during the filter loading period.

The filter installed in each furnace was a pleated 16" x 25" x 1" Filtrete Micro Allergen Extra Reduction Filter (3M, St. Paul, MN). During the data collection period (May 25 to May 28) air samples were taken on eight occasions at home 1 and on seven occasions at homes 2 and 3. On each occasion two side by side samples were collected onto MEA plates with an Andersen single stage viable impactor at two locations in the home and one location outside. The indoor locations were the parlor area and the basement. Outdoor samples were taken immediately adjacent to each home. All outdoor samples were taken for 5 minutes while indoor samples were taken for 10. The only exception to this was that the basement samples in home 3 were taken for 5 minutes to avoid overloading the agar plates. Velocity measurements were taken in the return duct to calculate flow rate. For the purposes of airflow calculations, an effective filter surface area exposed to airflow of 307 in^2 (13.5" x 22.75) was measured for the filters.

Table 4.3 Comparison of Home Air Handling Units

Home	Airflow (CFM)	Face Velocity (FPM)	Total Air Volume through Filter (m^3)	IAQ Concerns	Basement	Pets
1	1306	612	1.69×10^5	minor	semi-finished	dog
2	1640	769	2.15×10^5	no	finished	none
3	1353	634	1.69×10^5	serious	unfinished w/ dirt floor	dog

Filter quantification of these filters was performed in a manner identical to the 2 week and 1 week outpatient facility filters described previously. As in the outpatient facility, the three homes were ranked with respect to the relative airborne fungal concentration expected to be discovered in each home. This ranking was based on occupant symptoms and concerns, the level to which the basement was finished and the presence or absence of pets commonly associated with allergy symptoms. In taking these factors into consideration it was determined that home 3 would have the highest expected airborne concentrations while home 2 would have the lowest expected concentrations.

Administrative Facility

The administrative facility consisted primarily of office and meeting space on each floor. The building is served by a single HVAC system consisting of a ten filter bank. Eight of the ten filters were 24" x 24" x 2" Facet FME 40 Medium Efficiency Pleated Filters, and two were 12" x 24" x 2" Flanders PrecisionAire Pre Pleat filters (Flanders~PrecisionAire, St. Petersburg, Fl). In this system, these filters served as pre-filters for higher efficiency bag-type filters. Building administrators did not have accurate records of system operation and as a result, air velocity measurements were taken at the

center of each test filter in the bank and were used to calculate an estimate of air flow through the entire bank. The HVAC system in this facility operated continuously (24 hours/day). Figure 4.1 is a representation of a head-on-view of the filter bank, with each box representing a filter. The 12" x 24" filters are represented by the smaller boxes on the right. Each filter is numbered and contains the air velocity measurement taken at the filter's center.

1 (345 FPM)	2 (440 FPM)	3 (350 FPM)	4 (340 FPM)	9 (355 FPM)
5 (335 FPM)	6 (370 FPM)	7 (375 FPM)	8 (355 FPM)	10 (373 FPM)

Figure 4.1 Head-On-View of Administrative Facility Filter Bank with Filter Number Designations and Velocity Measurements.

Air flow through the entire bank was estimated by multiplying the average of the air velocity measurements (364 FPM) by the total surface area exposed to flow for all filters combined. Each 24" x 24" filter was measured to have an area exposed to flow of 473 in2 (21.75" x 21.75"). The area exposed to flow for the 12" x 24" filters was half of this value (236.5 in^2). The airflow estimated in this manner was 10,760 CFM. This value corresponds to airflow of 1196 CFM through each 24" x 24" filter and a value of 598 CFM through each 12" x 24" filter.

The facility had a history of water leaking in through the foundation into the basement. The problem had been addressed at various times over the three years prior to

the commencement of data collection and is currently less severe than it initially was; however occasions of heavy rain continued to cause foundation leaks into the basement area. Indoor air quality complaints have been reported on an infrequent basis by occupants of the basement offices, but not by occupants on the upper floors.

Data collection began in this facility on March 5, 2004 and continued until April 20, 2004. During this time period sampling was conducted in each of two areas of the building basement on ten separate occasions. On each occasion, two side by side samples were taken onto MEA with Andersen single stage viable impactors in each of the two areas. Area 1 was a hallway in the vicinity of offices housing individuals who most often filed complaints. This area was served directly by the HVAC system and had both active supply and return air vents. Area 2 was an elevator lobby with 4 doors off of it leading to a stairway, a meeting room, the previously described hallway, and a restroom. This area was not supplied directly by the ventilation system and had only active return air vents. Outdoor samples were taken on the final five sampling collection days. All indoor samples were collected for 15 minutes while all outdoor samples were collected for 10 minutes.

During the data collection period, two sets of test filters were loaded and quantified. On each of the occasions during which test filters were installed, the entire filter bank was replaced. Used filters were removed and new filters installed on March 5, April 13, and April 20. This schedule resulted in one filter being loaded for approximately 6 weeks (40 days, 2×10^6 m^3 air through each individual filter) and another for approximately 1 week (8 days, 3.88×10^5 m^3 air through each individual filter).

Filter quantification involved the previously described procedure with the following modifications with respect to the number of filter sections removed and the volume of shaking solution. Each of the quantified 24" x 24" filter had 5 sections manually removed and placed in 100 ml of 0.9% sterile saline with 0.1% TWEEN-20 in 250 ml Erlenmeyer Flasks. Quantified 12" x 24" filters had 3 sections removed. In both cases each of the filter sections was 2.25" wide and three pleats long. Figure 4.2 displays a photograph of a filter sample.

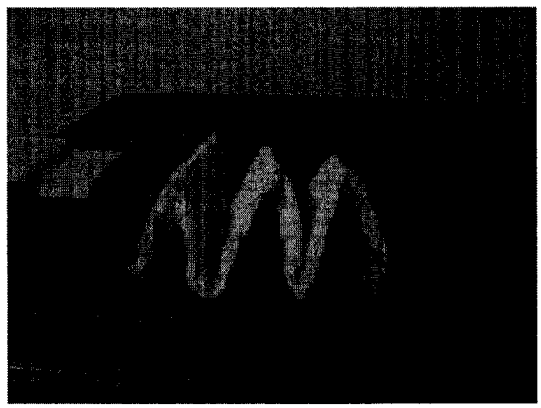

Figure 4.2 Pleated Filter Sample

The first set of filters that underwent quantification were the 6 week filters (removed April 13). Five of the filters in the filter bank that were loaded during this six week period (filters 5, 2, 4, 7 and 10) had the quantification procedure performed on them. The second set of filters quantified were the 1 week filters (removed April 20). Three filters (6, 8, and 3) were quantified from this group. In both of these cases, filters were chosen in a manner that allowed for a representative sample from the bank.

Research Facility

The research facility consisted primarily of classrooms, administrative offices, and laboratories. There was no history of indoor air quality complaints or water damage or leakage associated with this facility. This building is served by several large HVAC systems, two of which were involved in this research. Both of these units are located on the fourth floor in the same mechanical room and each serve one half of both the second and third floors. Unit 1 serves the eastern half of floors two and three, while unit 2 serves the western half of these floors. The two HVAC system filter banks were designed identically with a design air flow of 19,000 CFM and a 10 filter bank (1900 CFM/filter). All ten filters in each of the units were 24" x 24" x 2" Facet FME 40 Medium Efficiency Pleated filters. As in the administrative facility, these filters served as pre-filters for higher efficiency bag-type filters. Both systems were operated via computer and programmed to operate from 7:00 AM to 11:00 PM Monday through Friday. This operating schedule resulted in a total air volume of 1.5 x 106 m^3 through each filter over the data collection period. Figure 4.3 is a representation of a head-on-view of the filter bank, with each box representing a filter. Each box contains the number assigned to that filter.

1	2	3	4	5
6	7	8	9	10

Figure 4.3 Head-On-View of Research Facility Filter Bank with Filter Number Designations

Data Collection began in this facility on March 8, 2004 and continued until April 20, 2004 (44 days, approximately 6 weeks). Filters were removed on April 16, 2004. During this time period sampling was conducted in each of two areas of the building on 10 separate occasions. On each occasion, two side by side by samples were taken onto MEA with Andersen single stage viable impactors in each of the two areas. The two locations in which sampling was performed were hallways on the western side of floor two (designated as west and served by unit 2) and the eastern side of floor three (designated as east and served by unit 1). These locations were considered representative of the entire area served by each, thus the second floor sample was representative of the western sides of floors 2 and 3, while the third floor sample was representative of the eastern sides of floors 2 and 3. Outdoor samples were taken on all but the first (March 9) sampling occasion. All samples were taken for 15 minutes except for the final five outdoor samples which were taken for 10 minutes.

Filter quantification was performed through the use of the same method applied to the administrative building filters. Filters 2, 8 and 5 from both banks were quantified.

Summary

Table 4.4 below summarizes the filter loading periods in all facilities and details the number of air samples taken during each of these loading periods.

Table 4.4 Summary of Filter Loading and Air Sampling Procedures Performed in All Facilities

Facility and Filter Loading Periods	Number of HVAC systems	Filter Loading Period	Number of Air Samples Taken During Loading Period[1]
Outpatient Facility			
period 1	5	7 weeks	11
period 2	5	2 weeks	4
period 3	5	1 week	2
Residential Facilities			
period 1	3	4 days	7 or 8
Administrative Facility			
period 1	1	6 weeks	8
period 2	1	1 week	2
Research Facility			
period 1	2	6 weeks	9

1. In duplicate

Results

In order to assess the strength of association between filter quantification and air sampling results, two different regression procedures were performed on the collected data in which filter quantification and air sampling data were used as the dependent and independent variables respectively. In the first of these methods a single average filter quantification value (CFU/m^3) was plotted against each of the sampling concentrations (an average of the two side by side measurements in CFU/m^3) obtained while the filter was in service. In this analysis the number of air sampling values plotted against each

filter quantification value represents the actual number of air samples taken while that filter was in service. For example, as presented in Table 4.4, outpatient facility period 2 (2 week) would have been plotted against the results of each of the 4 individual air samples performed while the filter was in service.

Through these analyses it was discovered that the variance of model error was not constant and that the residuals were not normally distributed. A Box-Cox (SAS v8.02) procedure performed on the data indicated that the appropriate measure necessary to control this type of data set was to perform a $\log_{10}$ transform of the air sampling concentration averages for the purpose of statistical comparison. This transformation successfully controlled the unequal variance and error normality issues.

The second regression procedure performed to assess the strength of association between the air sampling and filter quantification involved the plotting of a single average filter quantification value (CFU/m^3) against the average of all the air sampling measurements taken in the area served by the filter while the filter was in service. For example, as presented in Table 4.4, outpatient facility period 2 (2 week) would have been plotted against the single average value of the 4 air samples performed while the filter was in service. In these procedures there were no significant normality or variance issues associated with the error terms and as a result the air sampling data did not require transformation.

Outpatient Facility

A regression performed with filter quanFtification and the logvalues of the air sampling concentrations for all outpatient facility loading periods as the dependent and independent variables respectively, indicated the presence of a statistically significant relationship between these two variables (SAS v8.02, PROC REG, p=0.010, R^2=0.08). The same analysis performed with the untransformed average air sampling values also indicated the presence of a statistically significant relationship (SAS v8.02, PROC REG, = 0.002, R^2=0.52). A summary of the data on which these analyses were performed is presented in Table 4.5. Appendix Table B.1 contains detailed sampling times and date information as well as CFU counts and the resulting concentration in cfu/m^3.

A comparison of Figures 4.4 and 4.5 demonstrates the similarities in relative air concentrations between filter quantification and air sampling leading to the finding of a statistically significant relationship. In order to ensure that covariance between filter quantification and duration of filter loading did not adversely influence the regression results, analysis of covariance (ANCOVA) was performed on the log transformed data (SAS, v8.02, PROC GLM) to determine if the quantification results varied significantly between the 7 week, 2 week and 1 week loading times. The results indicated a significant difference in filter quantification results between the 7 week and 1 week loading time (p=0.0002), and between the 2 week and 7 week loading time (p<0.0001) but not between the 1 week and 2 week (p=0.059) loading times. These results are summarized in Table 4.6. Analysis of variance (SAS v8.02, PROC GLM) of the ratios of the average air sampling values to filter quantification values presented in Table 4.5, with loading

period as the independent variable, indicated that the ratios were higher (p=0.002) for the 7 week loading period (mean=53.19) than for the 2 week (mean=24.19), or the 1 week (19.44) loading periods. The one week and two week periods were not significantly different.

Table 4.5 Summary of Air Sampling and Filter Quantification Results for All Time Periods at Five Outpatient Locations

Quantification Period / Location	Air sampling mean concentration (CFU/m^3)	Mean of Log_{10} Transformed Air Concentrations	Filter quantification mean detected concentration (CFU/m3)[1]	Air sample/ filter quantification	Filter Coefficient of Variation (s/mean)
7 week / area 1	69.62	1.75	1.3	54.88	0.233
7 week / area 2	52.60	1.63	1.07	52.77	0.187
7 week / area 3	51.99	1.58	0.80	57.56	0.341
7 week / area 4	34.99	1.50	1.32	28.25	0.010
7 week / area 5	66.21	1.61	0.96	72.47	0.322
2 week / area 1	134.18	2.04	7.23	18.56	0.237
2 week / area 2	45.54	1.61	2.39	19.05	0.297
2 week / area 3	45.63	1.63	1.02	44.74	0.173
2 week / area 4	34.26	1.50	3.2	10.71	0.299
2 week / area 5	65.21	1.76	2.34	27.87	0.274
1 week / area 1	92.84	1.88	3.52	26.38	0.355
1 week / area 2	25.32	1.40	1.81	13.99	0.229
1 week / area 3	45.47	1.58	1.76	25.84	0.219
1 week / area 4	25.90	1.41	1.45	17.86	0.211
1 week / area 5	29.64	1.43	2.26	13.12	0.230

1. Concentration extrapolated from CFU/plate, filter sample size, filter size, airflow rate, and unit run time. Equation (equation 2.1) $[(CFU/PV)*SV*FSA/SSA]/A_{total}$ Where CFU=colonies on plate, PV=plating volume (0.1mL), SV=shaking volume (50 ml or 75 ml), FSA=total filter surface area, SSA=filter sample surface area , A_{total}=total airflow through filter

Table 4.6 Summary of ANCOVA Analysis Performed to Determine if Filter Quantification Varied Significantly Between Three Different Filter Loading Durations

Comparison	P value
1 week vs 2 week	0.059
2 week vs 7 week	<0.0001
1 week vs 7 week	0.0002

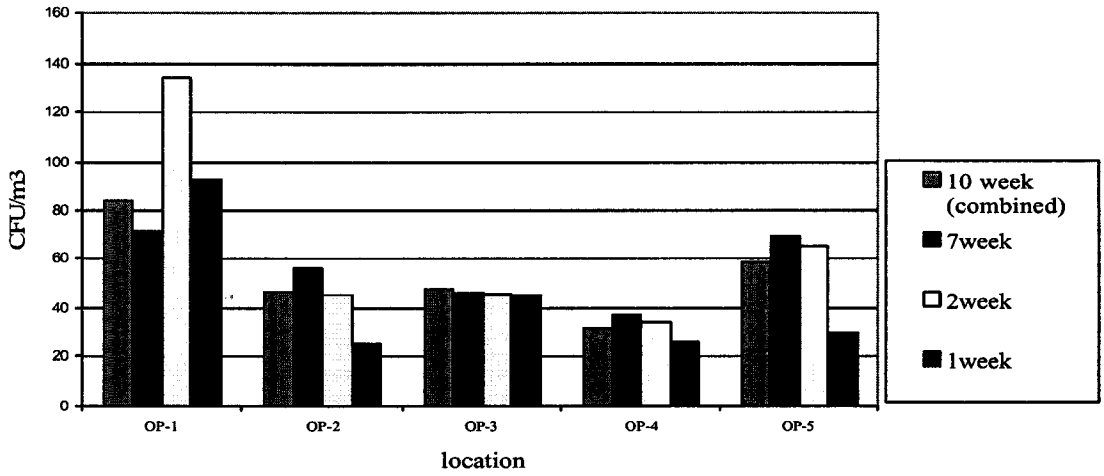

Figure 4.4 Outpatient Facility Average Air Sampling Results

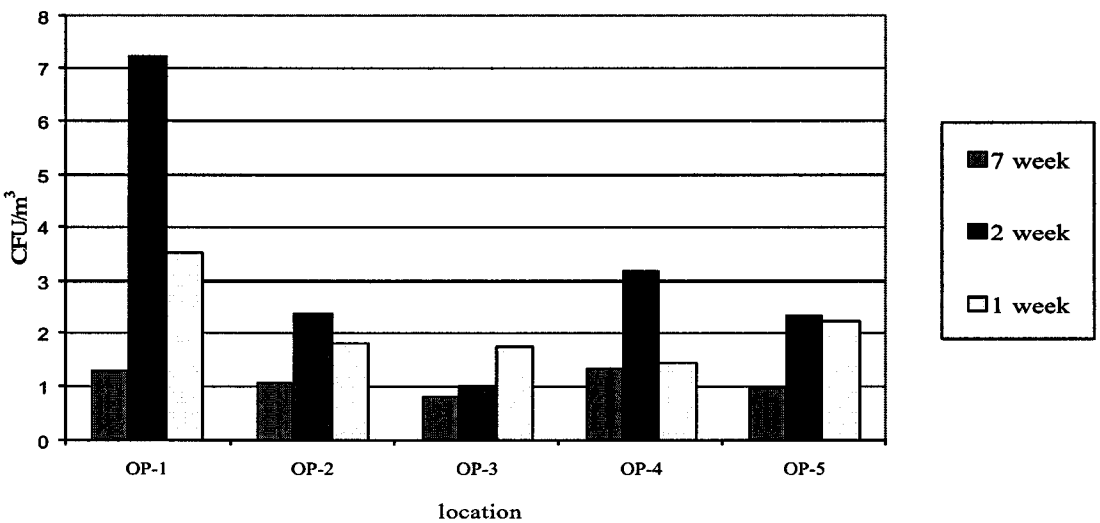

Figure 4.5 Outpatient Facility Average Filter Quantification Results

Due to the significant covariance between loading period and filter quantification, a clearer picture of the relationship between filter quantification and the transformed air sampling averages was drawn by comparing the results of the two sampling procedures (filter quantification and air sampling results) within each loading period. These comparisons were made by running individual regressions (SAS v8.02, PROC REG) with filter quantification and $\log_{10}$ transformed air sampling values or untransformed average air sampling values as the dependent and independent variables. The results of the log transformed data analysis indicated that a statistically significant relationship existed between the variables for the one week ($p=0.050$) and the two week ($p=0.024$) data. The results of the untransformed data analysis also indicated the presence of a statistically significant relationship between the one week ($p=0.037$) and the two week ($p=0.047$). Table 4.7 summarizes the results of these regressions. Figures 4.6 and 4.7 display plots of the significant transformed data results. Figures 4.8 and 4.9 display plots of the significant untransformed data results.

Table 4.7 Summary of Outpatient Facility Regressions Analyses Performed on Filter Quantification Results versus Corresponding Air Sampling Results

Regression	Significant (Y/N)	p-value	R^2
Log_{10} Transformed Analysis			
one week filter vs one week air samples	Y	0.050	0.40
two week filter vs two week air samples	Y	0.024	0.25
seven week filter vs seven week air samples	N	0.788	0.00
Untransformed Analysis			
one week filter vs one week air sampling average	Y	0.037	0.81
two week filter vs two week air sampling average	Y	0.047	0.78
seven week filter vs seven week air sampling average	N	0.786	0.03

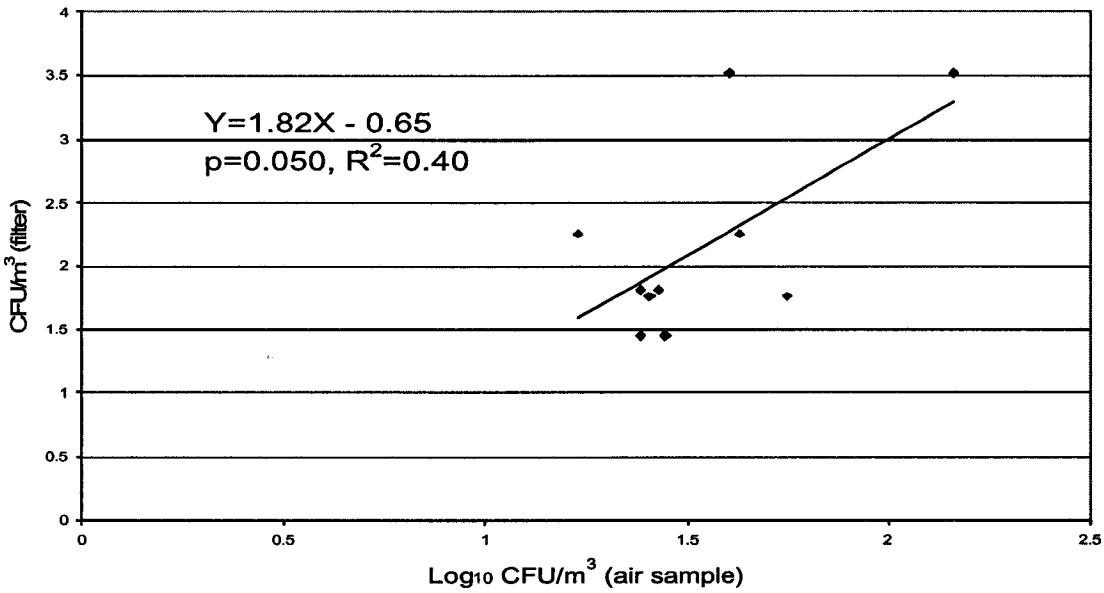

Figure 4.6 One Week Filter Quantifications vs. Log_{10} One Week Air Sampling Results (outpatient data)

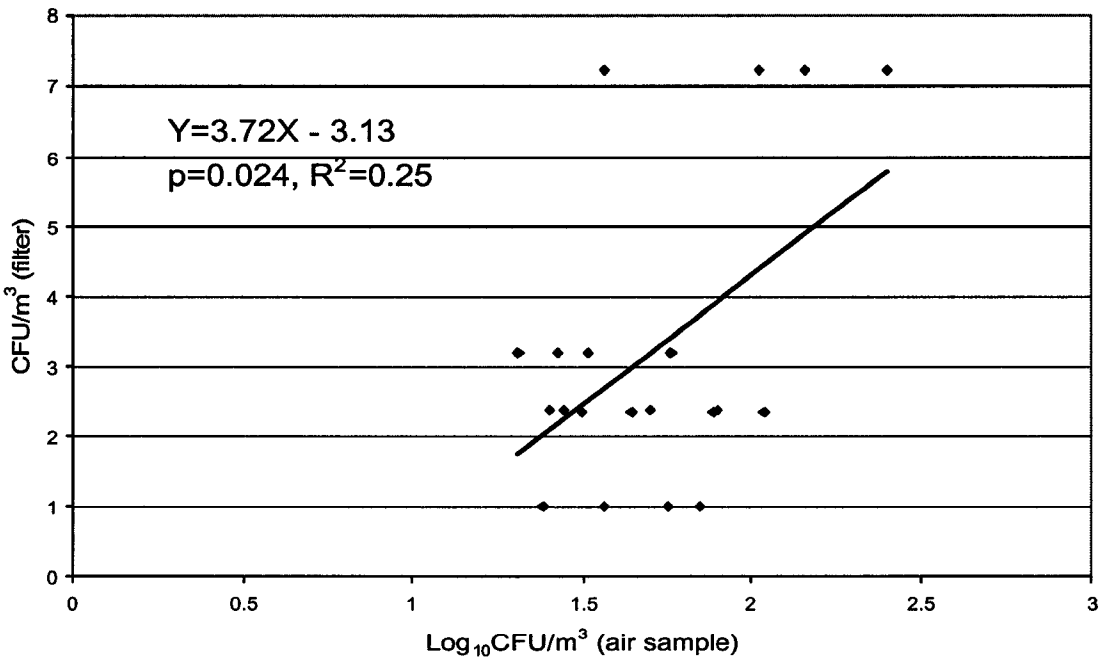

Figure 4.7 Two Week Filter Quantification vs. Log_{10} Two Week Air Sampling Results (outpatient data)

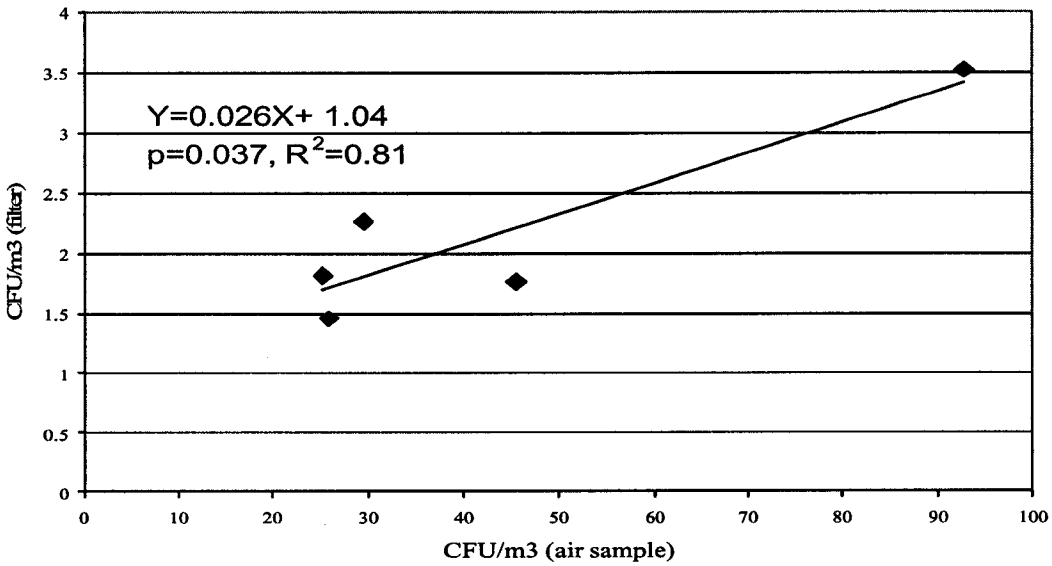

Figure 4.8 One Week Filter Quantification vs. One Week Average Area Air Sampling Value (outpatient data)

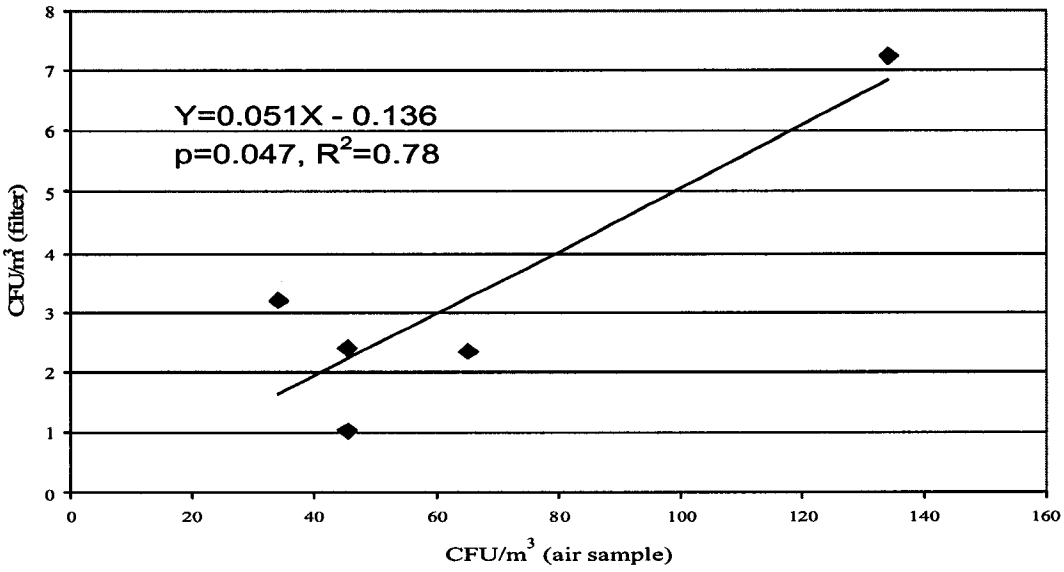

Figure 4.9 Two Week Filter Quantification vs. Two Week Average Area Air Sampling Value (outpatient data)

In addition to regression analysis performed to characterize the strength of the relationship between filter quantification and the $\log_{10}$ transformed average air sampling results, an analysis of variance (SAS v8.02, PROC GLM) was performed to compare the ability of the two methods (air sampling and filter quantification) to differentiate between the five areas with respect to amount of viable airborne fungal particle presence. In this analysis the filter comparisons involved the inclusion of each individual filter sample result (6 or 7 from each filter), while the air sampling comparisons involved the inclusion of each individual air sampling concentration result. The air concentrations for each area were compared to the expected relative levels (expected descending concentration order 1,5,3,2,4) based on the information in Table 4.1. Based on the criteria used to determine expected relative concentrations for the five areas, it was expected that at minimum, a significant difference should exist between areas 1 and 4. Each of the filter quantification result comparisons determined there to be at least one significant difference between area concentrations. A significant difference using the air sampling data was identified only when all ten weeks of the air sampling results (over all three loading periods of 1, 2, and 7 weeks) were averaged. Table 4.8 presents a summary of the results of these ANOVA procedures.

Table 4.8 ANOVA Results of Outpatient Facility Filter Quantification and Air Sampling Results

Sample	Statistically Significant Difference Between at Least Two Areas (Y/N)	P-value for Significant Difference Between at Least Two Areas	Order (descending)[1]	Grouping[2]
10 week (all samples combined)	Y	0.016	1,5,3,2,4	A: 1 AB: 2,3,5 B: 4
7 week filter	Y	0.0098	4,1,2,5,3	A: 1,4 AB: 2,5 B: 3
7 week air	N	0.495	1,5,2,3,4	A: 1,5,2,3,4
2 week filter	Y	<0.0001	1,4,2,5,3	A: 1 B: 4 BC: 2,5 C: 3
2 week air	N	0.074	1,5,3,2,4	A: 1,5,3,2,4
1week filter	Y	0.0001	1,5,2,3,4	A: 1 B: 5,2,3,4
1 week air	N	0.359	1,3,5,4,2	A:1,3,5,4,2

1. Individual HVAC units/areas presented in descending order of quantification values
2. Groups not sharing letters are significantly different from one another. Groups sharing a letter are not statistically significant from one another.

Residential Dwellings

Regressions performed with filter quantification and log transformed air sampling results as the dependent and independent variables respectively indicated that a statistically significant relationship did exist between both basement (p=0.003) and first floor (p=0.018) filter quantification and log transformed air sampling results. No statistically significant relationship was found between filter quantification and log transformed average outdoor air sampling results (p=0.604). Regressions performed with

filter quantification and untransformed average air sampling results as the dependent and independent variables respectively did not indicate a significant relationship between filter quantification and first floor air sampling averages (p=0.182), basement air sampling averages (p=0.325), or outdoor air sampling averages (p=0.789). A summary of the air sampling and filter quantification results is presented in Table 4.9. Appendix Table B.2 contains detailed sampling time and date information, as well as CFU counts and the resulting concentrations in cfu/m^3. The filter quantification coefficient of variation values presented in this table were calculated as described previously for the outpatient facility. In Table 4.9 the only filter to average air concentration ratios given are those for the first floor data. The rationale behind this is that the first floor samples were the only ones taken in an area directly served by the HVAC system in each home. The $\log_{10}$ transformed data outdoor air sampling data is not included in Table 4.9 due to the lack of statistical significance of these results. Table 4.10 displays the results of the regression analysis performed on this data. Figures 4.8 and 4.9 are plots of the of filter quantification versus log transformed first floor and basement air sampling values. Figures 4.10 and 4.11 are plots of the filter quantification versus untransformed first floor and basement air sampling averages.

Table 4.9 Residential Dwelling Filter Quantification and Average Air Sampling Summary

Home	Filter Quantification (CFU/m^3)[1]	First Floor Air Sampling Average (CFU/m^3)	Mean of Log_{10} Transformed First Floor Air Concentrations	Basement Air Sampling Average (CFU/m^3)	Mean of Log_{10} Transformed Basement Air Concentrations	Outdoor Air Sampling Average (CFU/m^3)	First Floor Air Sample / filter quantification	Filter Coefficient of Variation (s/mean)
1	19.37	393.73	2.57	386.91	2.54	1188.43	20.33	0.097
2	7.43	148.58	2.13	351.73	2.51	980.40	20.00	0.289
3	31.38	1154.28	2.61	3106.22	3.28	871.88	36.87	0.053

1. Concentration extrapolated fro m CFU/plate, filter sample size, filter size, airflow rate, and unit run time. Equation (equation 2.1): $[(CFU/PV)*SV*FSA/SSA]/A_{total}$ Where CFU=colonies on plate, PV=plating volume (0.1mL), SV=shaking volume (50 ml), FSA=total filter surface area, SSA=filter sample surface area , A_{total}=total airflow through filter

Table 4.10 Summary of Regression Analyses Performed on Filter Quantification Results versus Corresponding Air Sampling Results and Overall Air Sampling Results in Residential Dwellings

Regression	Significant (Y/N)	p-value	R^2
Log10 Transformed Analysis			
filter vs. basement air	Y	0.0003	0.49
filter vs. first floor air	Y	0.024	0.23
filter vs. outside air	N	0.604	0.01
Untransformed Analysis			
filter vs. basement air Sampling Average	N	0.325	0.76
filter vs. first floor air Sampling Average	N	0.182	0.92
filter vs. outside air sampling average	N	0.780	0.11

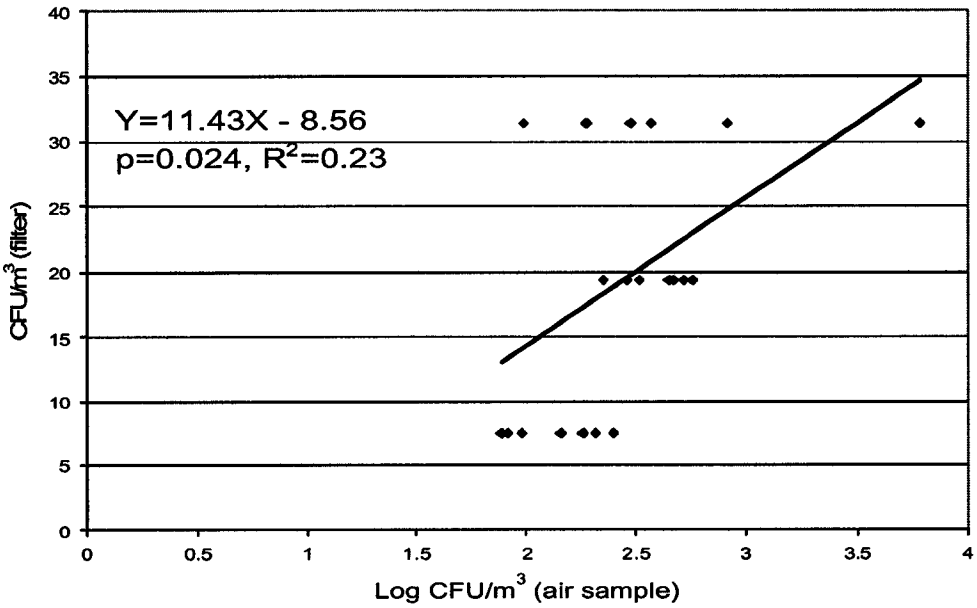

Figure 4.10 Filter Quantification vs. Log_{10} Transformed First Floor Air Sampling Results (home data)

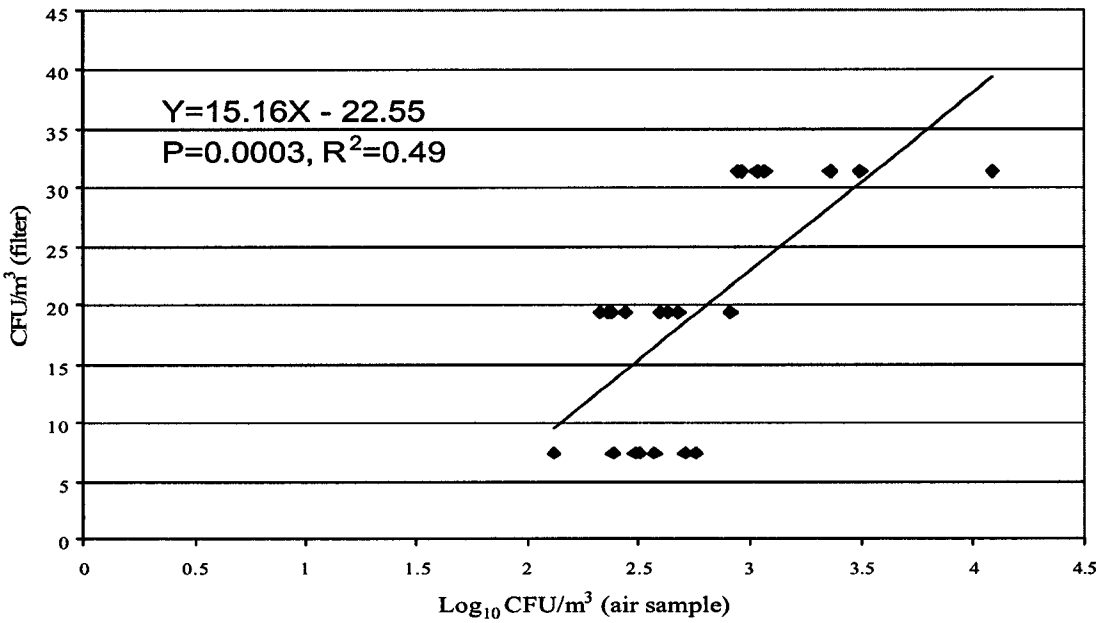

Figure 4.11 Filter Quantification vs. Log_{10} Transformed Basement Air Sampling Results (home data)

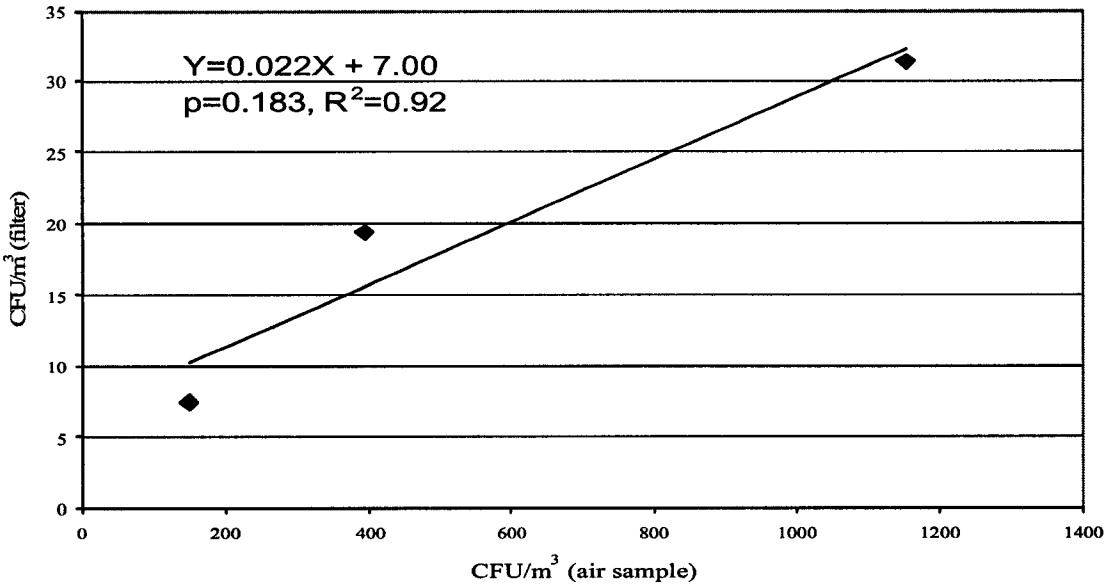

Figure 4.12 Filter Quantification vs. First Floor Average Air Sampling Value (home data)

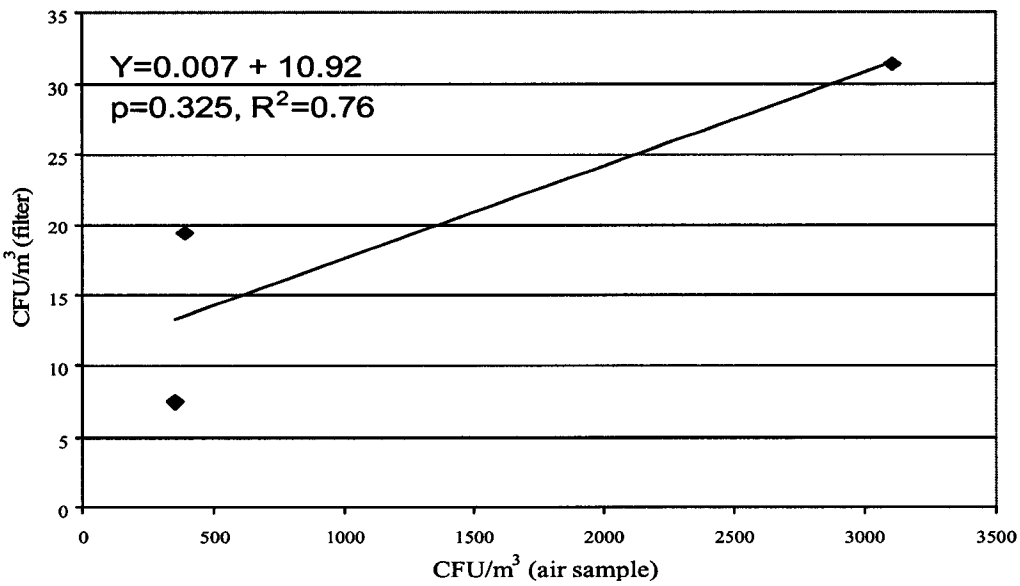

Figure 4.13 Filter Quantification vs Basement Average Air Sampling Value (home data)

Analysis of variance (SAS v8.02, PROC GLM), with location as the independent variable, was performed to compare the ability of the filter quantification and $\log_{10}$ transformed air sampling results to differentiate between the three homes with respect to quantity of viable airborne fungal particle presence. Filter quantification results identified a statistically significant difference between each of the three homes ($p<0.0001$). Log_{10} transformed results of both basement air ($p<0.0001$) and first floor air ($p=0.039$) samples also indicated a statistically significant difference; however these analyses separated the three homes into two groups and not three. No significant difference was indicated by analysis of $\log_{10}$ transformed results of outdoor air samples ($p=0.623$). The results of this analysis are summarized in Table 4.11.

Table 4.11 ANOVA of Filter Quantification and Air Sampling Results

Sample	Statistically Significant Difference Between at Least Two Areas (Y/N)	P-value for Significant Difference Between at Least Two Areas	Order (descending)[1]	Grouping[2]
Basement Air	Y	<0.0001	3, 1, 2	A: 3 B:1,2
First Floor Air	Y	0.039	3, 1, 2	A: 3,1 B: 2
Outdoor Air	N	0.623	1, 3, 2	A: 1, 2, 3
Filter Quantfication	Y	<0.0001	3, 1, 2	A: 3 B: 1 C: 2

1. Individual HVAC units/areas presented in descending order of quantification value
2. Groups not sharing letters are significantly different from one another. Groups sharing a letter are not statistically significant from one another.

Combined Outpatient Facility and Home Results

The fact that the same HVAC unit types were in service in all three study homes as well as in all five outpatient facility units, in combination with the fact that the one and two week outpatient facility filters were loaded for short periods of time, allowed for the home and short term outpatient loading data to be combined for statistical analysis purposes. The home and outpatient facility HVAC units used 1" pleated filters that were from different manufacturers, but were similar enough to allow for comparisons. Two regressions were performed using each of the regression methods previously described (4 total regressions), with either the $\log_{10}$ transformed or untransformed average air sampling results as the independent variable, on the combined air sampling and filter quantification results for the outpatient facility and the first floor data from the three homes. The first of these regressions involved the one week outpatient facility data, while the second involved the one week and two week data. In these combined regression analyses the 7 week outpatient data were excluded due to the lack of a significant relationship between the air sampling and filter quantification results during this loading period (Table 4.7). A second reason for their exclusion were the analysis of covariance results (Table 4.6) indicating a significant difference in the transformed air sampling results between the 7 week and both the 1 week and 2 week results.

The results of these combined regression analyses indicated a statistically significant relationship between $\log_{10}$ transformed air sampling and filter quantification concentrations for the combined one week outpatient and first floor home data (SAS v8.02, PROC REG, $p < 0.0001$, $R^2=0.56$) (Figure 4.12) as well as for the combined one and two week outpatient and first floor home data ($p= 0.0001$, $R^2=0.59$) (Figure 4.13).

Regression analysis of the untransformed data also indicated statistically significant relationships for the combined one week outpatient and first floor home data (SAS v8.02, PROC REG, $p < 0.0001$, $R^2 = 0.92$) (Figure 4.14) as well as for the combined one and two week outpatient and first floor home data ($p < 0.0001$, $R^2 = 0.93$) (Figure 4.15)

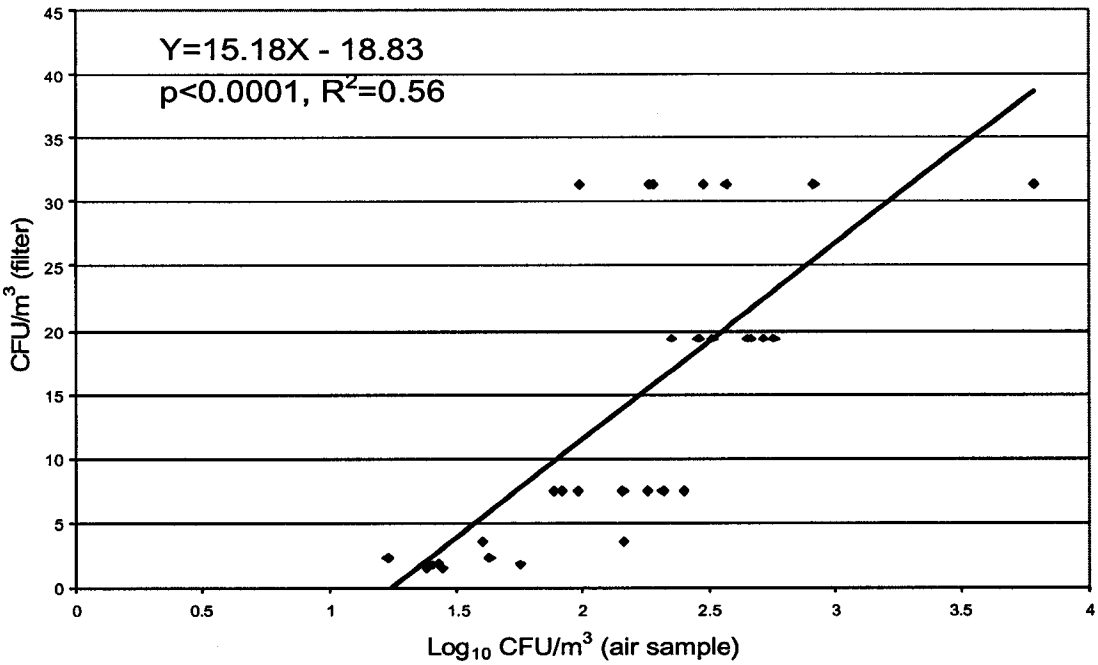

Figure 4.14 Filter Quantification vs. Log_{10} Air Sampling Results for Combined One Week Outpatient and First Floor Home Data

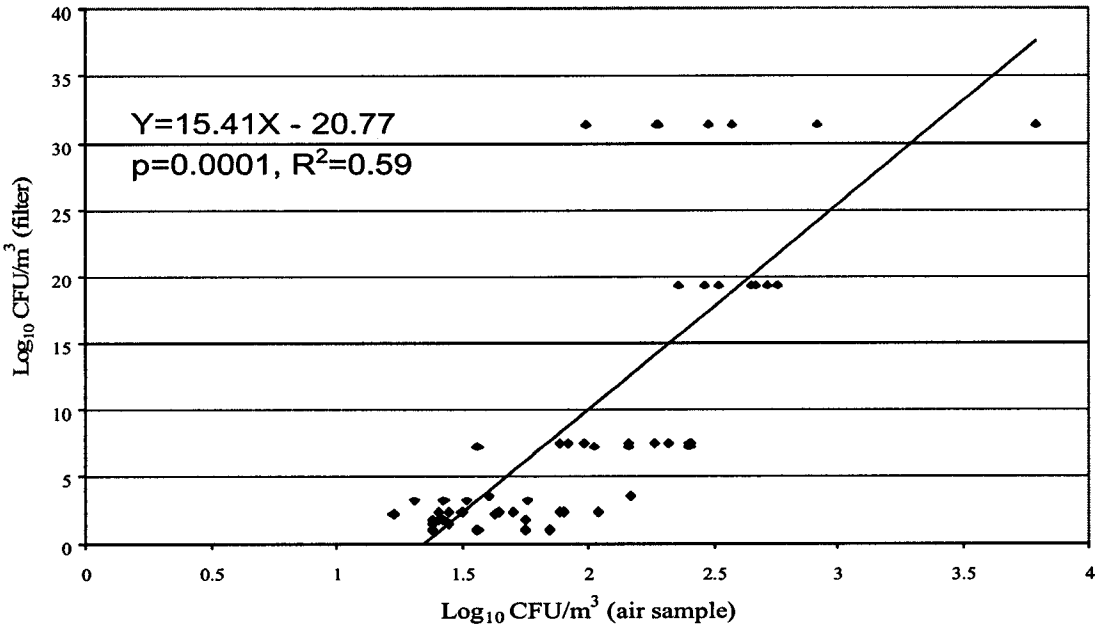

Figure 4.15 Filter Quantification vs. Log_{10} Air Sampling Results for Combined One and Two Week Outpatient and First Floor Home Data

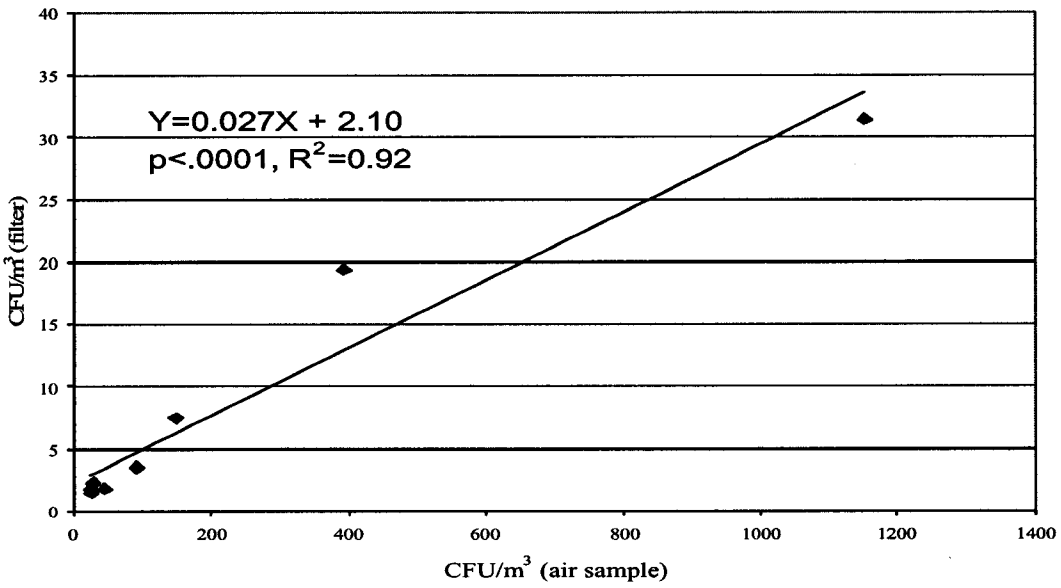

Figure 4.16 Filter Quantification vs. Average Air Sampling Value for Combined One Week Outpatient and First Floor Home Data

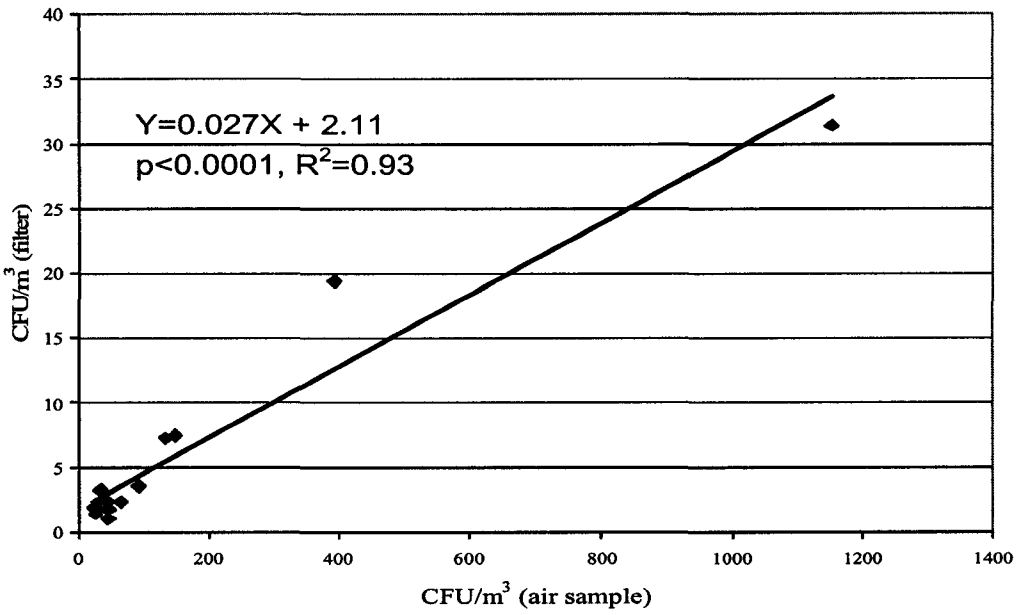

Figure 4.17 Filter Quantification vs. Average Air Sampling Value for Combined One and Two Week Outpatient and First Floor Home Data

Administrative Facility

The administrative facility contained only one HVAC system and thus comparisons between systems were not possible. Analyses involving the filter quantification data from this facility were performed by combining these results with the research facility results. The results of these analyses are presented in the following sections.

The average filter quantification results for the administrative facility were 3.56 cfu/m^3 and 3.55 cfu/m^3 for the one week and six week loading periods respectively. These results are summarized in Table 4.12. Appendix Table B.3 contains detailed sampling time and date information, as well as CFU counts and the resulting concentrations in cfu/m^3. The average air sampling concentration in this facility for all air samples taken (overall average) was 40.06 cfu/m^3. The overall air sampling

concentrations in areas 1 and 2 were 46.12 cfu/m^3 and 34.00 cfu/m^3 respectively. An ANOVA procedure performed on this data (SAS v8.02, PROC GLM), with area as the independent variable, indicated the lack of a significant difference with respect to the log transformed overall (7 week) average air concentrations between areas 1 and 2 (p=0.078). Six and 1 week period comparisons in transformed air concentration data between the two areas also resulted in the lack of statistically significant relationships (6 week p=0.121, 1 week p=0.748). A summary of the average air concentrations in this facility is displayed in Table 4.14. A summary of the ANOVA results is displayed in Table 4.15. Table 4.13 displays the mean filter concentrations and coefficients of variation for all administrative facility filters evaluated during the one and six week loading periods.

Table 4.12 Summary of Filter Quantification Results for the Administrative Facility

Period	Average Filter Concentration (cfu/m^3)[1]	Air sample[2] / filter quantification
1 week	3.56	11.22
6 week	3.55	12.20

1. Concentration extrapolated from CFU/plate, filter sample size, filter size, airflow rate, and unit run tim. Equation (equation 2.1): $[(CFU/PV)*SV*FSA/SSA]/A_{total}$ Where CFU=colonies on plate, PV=plating volume (0.1mL), SV=shaking volume (100ml), FSA=total filter surface area, SSA=filter sample surface area , A_{total}=total airflow through filter
2. Average air sample concentrationfor combination of both areas 1 and 2. (1week =35.93 cfu/m^3, 6 week=43.33 cfu/m^3)

Table 4.13 Mean Filter Quantification Results and Coefficients of Variation for Administrative Facility Loading Procedures

Loading Period and Filter	Mean Filter Concentration (CFU/m3)	Filter Coefficient of Variation (s/mean)
1 Week Loading		
Filter 6	3.39	0.156
Filter 8	3.75	0.296
Filter 3	3.54	0.262
6 Week Loading		
Filter 5	3.46	0.151
Filter 2	3.57	0.153
Filter 4	2.93	0.080
Filter 7	3.69	0.176
Filter10	4.08	0.039

Table 4.14 Summary of Average Air Sampling Results for the Administrative Facility

Period	Area	Average Concentration (cfu/m^3)	Average of Log_{10} Transformed Mean Air Concentrations
1 week	1	37.14	1.57
1 week	2	34.72	1.53
6 week	1	48.41	1.66
6 week	2	35.11	1.48
Overall (7week)	1	46.12	1.64
Overall (7 week)	2	34.00	1.48

Table 4.15 ANOVA Results for Average Air Sampling Concentration Differences between Administrative Facility Areas 1 and 2

Period	Significant (Y/N)	P value	Order (descending)[1]	Grouping [2]
1 week	N	0.748	1,2	A: 1,2
6 week	N	0.121	1,2	A: 1,2
Overall (7 week)	N	0.078	1,2	A: 1,2

1. Areas presented in descending order of average air sampling value
2. Groups not sharing letters are significantly different from one another. Groups sharing a letter are not statistically significant from one another

Research Facility

The average air sampling concentration for the eastern (served by HVAC unit 1) and western (served by HVAC unit 2) sections of the floors involved in the study were 31.85 cfu/m^3 and 20.09 cfu/m^3 respectively. The average filter quantification results for units 1 and 2 were 2.03 cfu/m^3 and 1.67 cfu/m^3 respectively for the single loading period of 6 weeks (Table 4.16). Table 4.17 displays the mean filter concentrations and coefficients of variation for all research facility filters. Appendix Table B.4 contains the details of sampling dates and times as well as cfu counts and resulting concentrations.

Table 4.16 Summary of Average Air Sampling and Filter Quantification Results for the Research Facility

HVAC Unit	Area Served	Average Air Sampling Concentration (cfu/m^3)	Mean of Log10 TransformedAir Concentrations	Average Filter Concentration (cfu/m^3)[1]	Air Sample / Filter Quantification
1	East	31.85	1.40	2.03	15.69
2	West	20.09	1.27	1.68	12.02

1. Concentration extrapolated from CFU/plate, filter sample size, filter size, airflow rate, and unit run time. Equation (equation 2.1) $[(CFU/PV)*SV*FSA/SSA]/A_{total}$ Where CFU=colonies on plate, PV=plating volume (0.1mL), SV=shaking volume (100ml), FSA=total filter surface area, SSA=filter sample surface area , A_{total}=total airflow through filter

Table 4.17 Mean Filter Quantification Results and Coefficients of Variation for Research Facility Loading Procedures

Area Served and Filter	Mean Filter Concentration (CFU/m3)	Filter Coefficient of Variation (s/mean)
East		
Filter 2	1.91	0.130
Filter 5	2.20	0.082
Filter 8	1.97	0.120
West		
Filter 2	1.66	0.124
Filter 5	1.64	0.122
Filter 8	1.73	0.090

Analyses of variance (SAS v8.02, PROC GLM), with area as the independent variable and $\log_{10}$ transformed average air sampling concentration as the dependent variable, performed to compare the ability of air sampling to differentiate between the eastern and western areas of floors 2 and 3 with respect to the viable airborne fungal concentration, indicated the lack of a significant difference between the two areas ($p=0.269$). Analysis of variance with filter quantification and area as the dependent and independent variables respectively, indicated a significant difference between the two areas ($p=0.0001$). Table 4.18 summarizes the results of these analyses.

Table 4.18 ANOVA of Research Facility Filter Quantification and Average Air Sampling Results

Sample	Significant (Y/N)	p-value	Floor Order (descending)[1]	Grouping[2]
Air	N	0.269	East, West	A: East, West
Filter	Y	0.0001	East, West	A: West B: East

1. Floors presented in descending order of quantification value
2. Groups not sharing letters are significantly different from one another. Groups sharing a letter are not statistically significant from one another

Combined Administrative and Research Facility Results

As with the outpatient facility and home data, the similarities between the administrative and research facility systems allowed for analyses to be performed on their combined data. Regression analysis performed on this data indicated a statistically significant relationship between the $\log_{10}$ transformed air sampling (independent variable) and filter quantification results (SAS v8.02, PROC REG, $p=0.002$, $R^2=0.31$) (Figure 4.16). There was no statistically significant relationship indicated between the untransformed air sampling average values and the filter quantification results (SAS v8.02, PROC REG, $p=0.222$, $R^2=0.88$) (Figure 4.17). Table 4.19 displays the combined research and 6 week administrative facility results.

Table 4.19 Summary of Combined Research Facility and Six Week Administrative Facility Results

Filter Bank	Average Air Sampling Concentration (cfu/m^3)	Mean of Log_{10} TransformedAir Concentrations	Filter Concentration (cfu/m^3)[1]	Air sample / filter quantification
research facility west	31.85	1.40	2.03	15.70
research facility east	20.09	1.27	1.67	12.03
Administrative (6 week)	43.33	1.56	3.55	12.20

1. Concentration ext rapolated from CFU/plate, filter sample size, filter size, airflow rate, and unit run time Formula: $[(CFU/PV)*SV*FSA/SSA]/A_{total}$Where CFU=colonies on plate, PV=plating volume (0.1mL), SV=shaking volume (100ml), FSA=total filter surface area, SSA=filter sample surface area , A_{total}=total airflow through filter

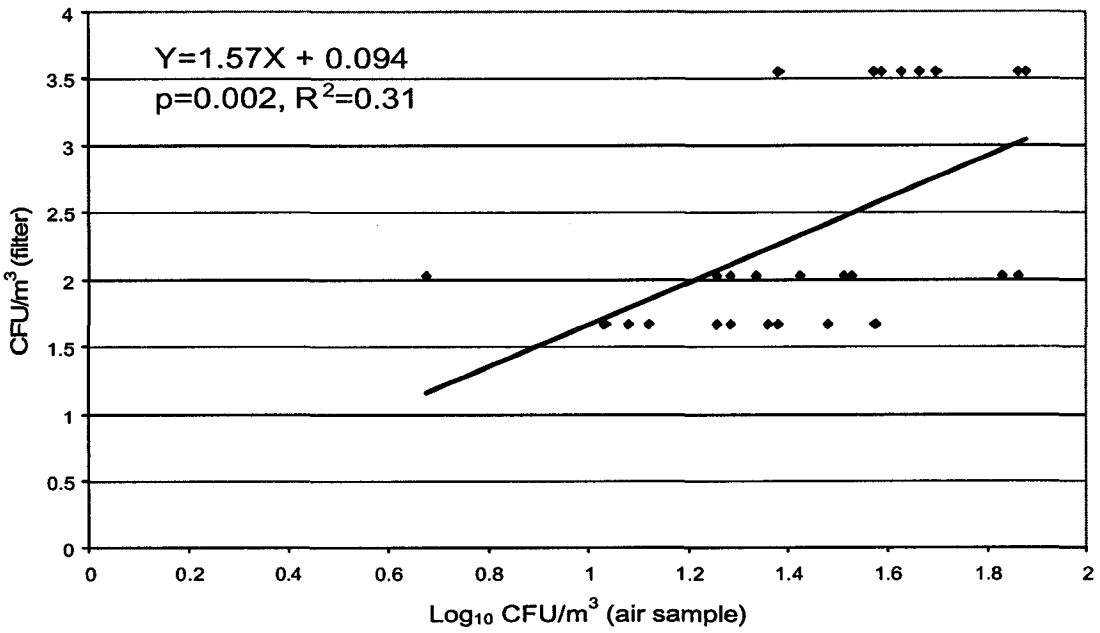

Figure 4.18 Filter Quantification vs. Log_{10} Transformed Air Sampling Results for Combined Research Facility and Six Week Administrative Facility Data

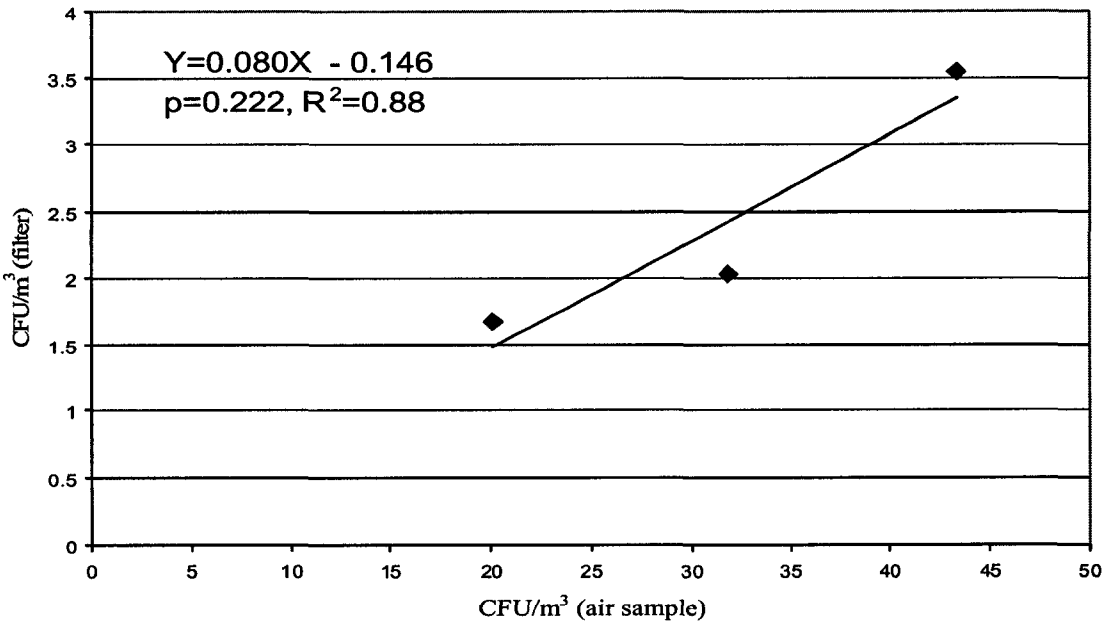

Figure 4.19 Filter Quantification vs. Average Air Sampling Values for Combined Research Facility and Six Week Administrative Facility Data

Discussion

This research involved the comparison of HVAC filter quantification and single stage impactor viable air sampling as tools used to assess viable fungal particle levels in indoor air. It is important to note that the purpose of the research was not to present filter quantification as a replacement for traditional short term viable sampling techniques, but as a potential tool to be used in conjunction with other assessment methods. Due to the short term nature of many viable air sampling techniques, their results often do not provide concentrations representative of long term air concentration averages. The involvement of the filter quantification method in indoor air quality investigations has the potential to improve the assessment of indoor airborne fungi over investigations performed using short term techniques alone by serving as a longer term integrated measure of relative levels between areas.

One of the primary strengths of the filter quantification method is that when compared the typical viable air sampling approach, a filter has a much larger quantity of air flowing through it per unit time. This fact in combination with comparatively much longer sampling periods (ie: 1 week or more vs 5 minutes) allows for an improvement in the time-integrated nature of filter sampling when compared to viable impactor sampling. The collection of samples onto growth media does not allow for individual samples to be collected for extended periods of time due to plate overloading and media desiccation. In order to increase confidence in air sampling results during indoor air quality investigations, numerous samples are often collected on various separate occasions with the intent of approximating a true average concentration. When this method is employed, as the number of samples increases, so does the confidence in the obtained average value.

In this research regular air sampling was performed while test filters were installed in test ventilation systems in order to obtain values in which the researchers were confident approximated the average air concentration of the tested areas. It was necessary to have accurate estimates of air concentrations with which to compare filter quantification values in order to evaluate the true nature of the relationship between the two sampling methods. In this research two methods were employed to evaluate the relationship between short term air sampling and filter quantification. The first method involved the comparison of filter quantification results with each individual air sampling value (log transformed) obtained in the area served by the filter while the filter was in service. The second method involved the comparison of filter quantification results with the average air sampling concentration value calculated from all the sampling results collected in the area served by the filter while the filter was in service. The strength of the individual air sampling comparison is that it allowed for the variability of the air sampling values in each area to be incorporated into the regression analysis. The average air sampling values have the advantage over the individual measurements of being more representative of the actual air concentration during the entire filter loading period. This fact makes them more similar (than any individual air sampling result) to the filter quantification results, which are integrated over the entire duration of filter service life.

Due to the increase in air sampling result confidence associated with increased sample numbers, it may be expected that filter quantification values would be more strongly associated with longer term rather than with shorter term air sampling values and averages. The outpatient facility results however, do not support this assumption. An examination of Table 4.7 demonstrates that the relationship between air sampling and

filter quantification results is most significant for the one and two week sampling periods for both the log transformed and the untransformed average data. Significance was not present with the seven week period in either analysis. A possible explanation for this phenomenon is related to the fact that as filter loading time increases, filter cake also increases. It was observed that when filters contained large amounts of filter cake, substantial amounts of dust were lost while processing the samples, thus losing substantial numbers of spores as well. Filters with shorter loading times were not found to have this problem associated with them. An examination of Table 4.5 demonstrates that the air sample to filter quantification ratios were higher for the seven week filters than for the 1 or 2 week filters. Analysis of variance (SAS, v8.02, PROC GLM), with loading period as the independent variable indicate that the 7 week average ratio value (53.23) was significantly greater (p=0.002) than both the 1 week (mean=19.44) and the two week (mean=24.19) values. This significantly higher ratio at the longer loading period, in combination with the lack of a statistically significant relationship, is consistent with the loss of viable particles from the filters. Also, two of the three home filter (homes 1 and 2) to average first floor air concentration ratios, 20.33 and 20.00, were similar to the outpatient one week value, indicating consistent results across the one week values.

Long term loading was not performed in the study homes, however the short term sampling and filter quantification results did indicate the presence of a statistically significant relationship between filter quantification results and each of both the $\log_{10}$ transformed first floor (p=0.024) and $\log_{10}$ transformed basement (p=0.0003) air sampling results. Analysis of the untransformed average data did not indicate a

statistically significant relationship between filter quantification results and either first floor ($p=0.183$) or basement ($p=0.325$) average air sampling value, however, the R^2 value of 0.92 and an examination of the data pattern in Figure 4.12 indicate the presence of a strong positive trend between filter quantification and first floor air sampling average. This trend is less evident with filter quantification and basement air sampling average ($R^2=0.76$, Figure 4.13). When the first floor transformed home air sampling values were combined with the transformed outpatient one week data, a significant relationship was found between the log transformed air sampling results and filter quantification ($p<0.0001$, $R^2=0.56$). The results from addition of the two week outpatient data also indicated a significant relationship ($p=0.0001$, $R^2=0.59$). The same analyses performed on the untransformed data also indicated statistically significant relationships for the combined one week outpatient and home data ($p<0.0001$, $R^2=0.92$) and the combined one and two week outpatient and first floor home data ($p<0.0001$, $R^2=0.93$). In both the transformed and untransformed analyses, the addition of the two week data did not significantly increase the R^2 value, suggesting that the addition of the two week outpatient data does not add significantly to the statistical model.

While the outpatient facility results indicated the lack of a significant relationship between the two sampling methods (filter vs air) at the longest loading period evaluated (7 week), the combined administrative and research facility log transformed data indicate the existence of a statistically significant relationship at a comparable (6 week) sampling duration ($p=0.002$, $R^2=0.31$, Figure 4.18). The same combined analysis performed on the untransformed average data did not indicate a statistically significant relationship ($p=0.222$, $R^2=0.88$), however an examination of Figure 4.19 and the R^2 value of 0.88

indicate the presence of positive trend. Due to the basic differences in design of the air handling systems and filters between the outpatient and home facilities and the administrative and research facility, the results may not be directly comparable, however the fact that the significant relationship existed in these larger systems may indicate that further research should not be limited to short term loading periods in all types of facilities, especially those with large filter banks. The loss of filter cake may not be as significant on the larger bank filters due to the larger surface area associated with increased filter pleat depth.

The finding in the outpatient facility, the homes, and the research facility that filter quantification analysis was more likely than air sampling analysis to identify statistically significant differences between areas (Tables 4.8, 4.11 and 4.18) points to a potentially important benefit of filter quantification analysis. In the field, this sensitivity would allow indoor air quality investigators to better assess the differences between areas and possibly help explain patterns in area-specific occupant symptoms and complaints, or perhaps even identify potential problem areas before symptoms and complaints occur.

Based upon the history of each area in the outpatient building it was assumed that area 1 would have the highest air concentrations, areas 5 and 3 would have significantly higher air concentrations than area 2 and that area 4 would have the lowest air concentration. Table 4.20 presents the expected concentration values and summarizes fungal concentration rankings for the outpatient facility areas. It is evident from the data summarized in Table 4.20 that air sampling agrees exactly with qualitative ranking for both the 2 week and 10 week air sampling values. The 7 week air sampling rankings varied from the expected rankings only in that the orders of areas 2 and 3 were reversed.

The ANOVA results presented in Table 4.8 also show that analysis of the 10 week air sampling data indicated a statistically significant difference between groups 1 and 4. Filter quantification order varied with the number of weeks the filter was in place, with 1 week being the best. The rankings resulting from the 2 and 7 week filter quantification results varied significantly from the expected order, however the 1 week filter ranking differed only in that the orders of areas 2 and 3 were reversed. For samples taken during the one week period, filter quantification statistically distinguished area 1 vs. area 4, whereas air sampling did not. These results suggest that short term filter loading may be more suitable than longer term loading for the purpose of comparing viable fungal air concentrations in different areas of a facility.

Table 4.20 Expected and Experimental Area Airborne Viable Fungal Particle Concentration Rankings for Outpatient Facility Areas 1 through 5

Expected Ranking Order (greatest to least)	Air Sample Ranking Order				Filter Quantification Ranking Order		
	1 week	2 week	7week	10 week	1 week	2 week	7 week
1	1	1	1	1	1	1	4
5	3	5	5	5	5	4	1
3	5	3	2	3	2	2	2
2	4	2	3	2	3	5	5
4	2	4	4	4	4	3	3

Conclusions

The primary conclusion drawn from this phase of the research is that a statistically significant positive relationship exists between air sampling concentrations and filter quantification values. The discovery of this relationship suggests that as viable

airborne fungal concentrations increase in an area, the filter quantification results will increase proportionately. A second conclusion is that while long term filter loading periods should not be dismissed in future research, short term loading periods appear to result in filter quantification results that are more strongly associated with air sampling average concentrations and also appear to be able to differentiate between potential problem and non-problem areas.

While this research has successfully demonstrated that filter quantification is a method that has strong potential for future use in the field, further study is needed to better characterize the nature of the relationship between actual air concentrations and filter quantification values. A limitation of this study was the small number (2) of air samples collected during the one week filter loading periods in the administrative and outpatient facilities. Due to measurement variability (see Appendix B) the number of samples taken may have been insufficient to accurately characterize the average air concentration over the filter loading period.

The involvement of the filter quantification method in indoor air quality investigations has the potential to improve the assessment of indoor airborne fungi over investigations performed using short term techniques alone by serving as a longer term integrated measure of relative levels between areas. While the integrated nature of the filter sampling technique is an important strength, the fact the method greatly underestimates air concentrations is a major weakness. This inability to determine actual concentrations limits the procedure's usefulness to relative comparisons between areas or within one area at different times. This limitation could be overcome if a relatively constant mathematical relationship between filter quantification and air sampling results

were to be found to exist for each different type of filter. The existence of this type of relationship would allow for average air sampling concentrations to be predicted from filter quantification results once these relationships have been independently validated through additional field research. Another method weakness is the apparent inconsistent relationship with long term air sampling averages. The lack of a relationship between filter quantification and air sampling results for the 7 week outpatient facility filters was at least partially due to the loss of filter cake during filter transport and processing. This problem could potentially be overcome through more careful handling of the filters in combination with the removal of filter samples at the filter bank; however limiting the filter quantification method shorter loading periods may be the best method to address this problem.

While there are significant weaknesses associated with the method, the results of this research do indicate that it has potential as a methodology for assessing airborne fungal levels. This conclusion is based on 1) the statistically significant relationships resulting from regression analyses of the study data, and 2) the high R^2 values seen in the regression analyses of the average air sampling results, although many of these analyses were not statistically significant. For example, the comparison of the log transformed first floor home data and filter quantification results indicates the presence of a statistically significant relationship between the two methods ($p=0.024$, $R^2=0.23$), however examination of the non-significant relationship in Figure 4.12 ($p=0.183$, $R^2=0.92$) more clearly displays the positive trend between first floor home air sampling and filter quantification results. This clearer representation of the relationship is also evident in the larger R^2 of the non-statistically significant analysis. In this example, and in all

regressions performed in this research, the R^2 was greater for the regression in which (non-transformed) average air concentration values were used as the independent variables (Figures 4.8, 4.9, 4.12, 4.13, 4.16, 4.17, 4.19). This result indicates that the average air concentration values are more strongly correlated with filter quantification results than with multiple individual air sampling values.

Reference

1. Macher, J., H.A. Amman, H.A. Burge, D.K. Milton, and P.R. Morey (eds): *Bioaerosols: Assessment and Control.* Cincinnati, Ohio: American Conference of Governmental Industrial Hygienists, 1999.

CHAPTER V

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

This research involved the laboratory testing and field evaluation of a method for the quantification of viable fungal particles on heating, ventilating and air conditioning (HVAC) system filters. One of the two main purposes of this research was to evaluate whether or not the filter quantification method is able to differentiate, with respect to viable fungal airborne levels, between areas suspected of having significantly different concentrations. This evaluation was performed in both chapters 2 and 4. In chapter 2 a statistically significantly greater number of mold spores was found on a complaint filter in comparison to a non-complaint filter from the same building and in service over the same period of time. In chapter 4 the filter quantification method for shorter loading periods (1 week) was found to produce results that were more likely than single stage impactor results to differentiate between areas suspected of having significantly different airborne fungal concentrations based on building history and occupant complaints. Chapter 4 results also suggested however, that an accurate ranking of relative concentrations (based on expected concentrations) appears to be possible at shorter (1 week) but not longer (2 or 7 week) loading periods. The second main purpose of this

research was to evaluate whether or not the concentration of viable fungal particles, as calculated through the filter quantification method, was significantly associated with the airborne concentration values obtained from the results of repeated single stage impactor sampling results collected while filters were in service. The results of this evaluation in buildings with residential style HVAC systems indicated a statistically significant association between filter quantification and air sampling results at shorter term (1 week and 2 week) loading periods, but not at a longer term (7 week) loading period. In contrast to the latter finding, sampling results of 6 week loading in buildings with larger HVAC units indicated a statistically significant relationship between air sampling and filter quantification results.

Chapter 3 involved the laboratory testing and development of the filter quantification procedure. The development of the procedure involved modifications designed to improve quantification efficiency. The addition of the surfactant TWEEN-20 to the filter shaking solution was found to significantly improve recovery efficiency and thus was incorporated into the quantification procedure performed on field filters in the research presented in chapter 4. The results of this research also indicated that nebulizers have the potential to become inexpensive and easy to use tools in bioaerosol research.

Conclusions

Based on the research presented in Chapters 2, 3 and 4, the following conclusions were reached

1. HVAC viable fungal particle filter quantification is a method that has strong potential for future use in indoor air quality investigations as a tool to assess relative levels of airborne fungal contamination.
2. A positive statistically significant relationship exists between filter quantification results and air sampling results taken while filters are in place for the data in this study.
3. The filter quantification method, applied to short periods, is sufficiently sensitive to distinguish between areas suspected of having significantly different levels of airborne fungal contamination.
4. Nebulizers are inexpensive and easy to operate tools for generating predictable air concentrations of fungal aerosols.

Recommendations

The following are recommendations for future research, based on the current findings:

1. The collection and recovery efficiencies of multiple filter types should be evaluated in order to determine which types of filters would be best suited for quantification.

2. The filter recovery efficiencies of multiple fungal genera should be evaluated.
3. Future field study should include an increase in the number of impactor (or other methods) samples taken during each filter loading period in order to obtain a more accurate estimate of average concentration.
4. The use of the filter quantification method as an epidemiologic tool to determine the relationship between mold exposure and occupant symptoms should be performed.

APPENDICES

Appendix A

Ventilation Test Chamber Air Velocity Data and Chamber Loading Procedure Raw Data

Tables A1 and A2 below present the air velocities in the test duct as measured with the one inch and two inch test filters in place. Velocity measurements were taken through small holes drilled through the outer vertical duct surface. Velocity was measured at four sampling locations along the horizontal length of the duct at 40", 76", 99" and 135" from the flow inlet. At each of these sampling location distances three holes were drilled at 6", 12" and 18" from the top horizontal surface of the duct. Measurements were taken at 6",12" and 18" from the drilled hole.

Table A.1 Feet Per Minute (fpm) Air Velocity Measurements Taken with 1" test filter in place (average velocity=381.9 fpm)

Verticle distance from top surface	40" from flow inlet			76" from flow inlet			99" from flow inlet			135" from flow inlet		
	6" from hole	12" from hole	18" from hole	6" from hole	12" from hole	18" from hole	6" from hole	12" from hole	18" from hole	6" from hole	12" from hole	18" from hole
6"	315	245	340	215	520	730	270	325	650	225	290	290
12"	305	260	315	310	310	970	365	325	560	250	265	265
18"	290	270	290	190	345	1110	240	415	810	245	285	345

Table A.2 Feet Per Minute (fpm) Air Velocity Measurements Taken with 2" test filter in place (average velocity=382.2 fpm)

Verticle distance from top surface	40" from flow inlet			76" from flow inlet			99" from flow inlet			135" from flow inlet		
	6" from hole	12" from hole	18" from hole	6" from hole	12" from hole	18" from hole	6" from hole	12" from hole	18" from hole	6" from hole	12" from hole	18" from hole
6"	265	270	285	280	660	630	310	410	450	260	300	305
12"	305	245	300	290	380	980	310	335	490	290	295	290
18"	305	225	295	240	315	1070	280	470	760	215	315	335

Tables A.3 through A.13 below present data for loading procedures performed under modification II conditions presented in Table 3.7. (TWEEN-20 added to harvesting fluid and shaking solution). Each box in table represents filter sample (top left, top center, top right, middle left, middle center, middle right, bottom left, bottom center, bottom right). Each comma separated value in each box represents the number of CFUs resulting from one replicate of plated filter sample shaking solution (0.2 ml plated). Pad filters had 9 samples removed while pleated filters had 5 samples removed.

Tables A.14 and A.15 present recovery efficiencies by filter section

A.3 *A. niger* Loaded onto Pad Filter with Expected average CFU Aalue=223 and Experimental Average CFU Recovery Value=135 (60% efficiency)

48, 64, 51	63, 63, 58	90, 87, 101
152, 121, 113	132, 159, 161	190, 160, 152
169, 144, 173	243, 209, 232	180, 173, 162

Table A.4 *A. niger* Loaded onto Pad Filter with Expected Average CFU Value=71 and Actual average CFU Recovery Value=95 (133% efficiency)

63, 69, 62	40, 69, 47	54, 29, 32
122, 106, 92	100, 98, 111	87, 98, 97
90, 118, 109	143, 147, 158	138, 145, 147

Table A.5 *A. niger* Loaded onto Pad Filter with Expected Average CFU Value=37 and Actual Average CFU Recovery Value=35 (93% efficiency)

31, 16, 20	20, 17, 11	18, 87, 14
42, 34, 26	42, 39, 38	42, 49, 30
23, 36, 31	38, 54, 43	57, 48, 37

Table A.6 *P. chrysogenum* Loaded onto Pad Filter with Expected Average CFU Value=106 and Actual Average CFU Recovery Value=64 (60% efficiency)

40, 32, 25	36, 15, 30	33, 26, 35
72, 73, 61	97, 89, 91	72, 50, 58
102, 88, 115	92, 98, 80	73, 68, 67

Table A.7 *P. chrysogenum* Loaded onto Pad Filter with Expected Average CFU Value=42 and Actual Average CFU Recovery Value=22 (53% efficiency)

9, 6, 9	5, 9, 15	8, 4, 7
38, 33, 30	25, 31, 26	35, 24, 18
40, 34, 35	31, 30, 36	22, 16, 25

Table A.8 *P. chrysogenum* Loaded onto Pad Filter with Expected Average CFU Value=34 and Actual Average CFU Recovery Value=26 (76% efficiency)

16, 13, 15,	16, 12, 17	12, 14, 21
22, 31, 41	33, 33, 19	35, 30, 24
25, 24, 22	37, 39, 33	32, 34, 59

Table A.9 *A. niger* Loaded onto Pleated Filter with Expected Average CFU Value=66 and Actual Average CFU Recovery Value=111 (168% efficiency)

80, 76, 65		50, 55, 50
	126, 115, 135	
136, 141, 112		194, 186, 149

Table A.10 *A. niger* Loaded onto Pleated Filter with Expected Average CFU Value=12 and Actual Average CFU Recovery Value=46 (383% efficiency)

36, 34, 21		15, 20, 11
	61, 64, 49	
71, 62, 67		65, 60, 53

Table A.11 *P. chrysogenum* Loaded onto Pleated Filter with Expected Average CFU Value=127 and Actual Average CFU Recovery Value=96 (76% efficiency)

53, 56, 42		69, 66, 54
	119, 99, 96	
116, 115, 115		153, 144, 139

Table A.12 *P. chrysogenum* Loaded onto Pleated Filter with Expected Average CFU Value=67 and Actual Average CFU Recovery value=38 (57% efficiency)

23, 23, 17		19, 19, 16
	38, 41, 61	
40, 49, 51		59, 50, 57

Table A.13 *P. chrysogenum* Loaded onto Pleated Filter with Expected Average CFU Value=38 and Actual Average CFU Recovery Value=76 (200% efficiency)

44, 45, 58		30, 43, 34
	87, 79, 70	
72, 102, 83		124, 136, 136

Table A.14 Recovery Efficiencies By Section for Pad Filters Loaded Under Modification Procedure II with TWEEN-20 added to Both the Harvesting Fluid and Shaking Solution

Horizontal Orientation[1]	Average[2]	Standard Deviation[2]	Range[2]
Top	54%	12%	38%-74%
Middle	114%	14%	97%-139%
Bottom	132%	11%	117%-146%
Vertical Orientation[1]			
Left	92%	10%	82%-106%
Middle	106%	6%	96%-112%
Right	101%	15%	84%-121%

1. Filters were broken up into thirds for the purposes of this analysis. In the horizontal orientation, top, middle and bottom refer to the three samples in each of the sections of the filters. The vertical orientation is broken up on the same manner.
2. For each filter, the average of each of the observed CFU values for each section was divided by the total observed CFU average for the entire filter. This value was then multiplied by 100%. The displayed percentages are the average, standard deviation, and range values for all six pad filters.

Table A.15 Recovery Efficiencies By Section for Pleated Filters Loaded Under Modification Procedure II with TWEEN-20 added to Both the Harvesting Fluid and Shaking Solution

Horizontal Orientation[1]	Average[2]	Standard Deviation[2]	Range[2]
Top	54%	4%	50%-60%
Middle	115%	10%	103%-126%
Bottom	134%	3%	136%-143%
Vertical Orientation[1]			
Left	92%	8%	87%-105%
Middle	115%	10%	103%-126%
Right	100%	12%	81%-110%

1. Filters were broken up into thirds for the purposes of this analysis. In the horizontal orientation, top, middle and bottom refer to the samples in each of the sections of the filters. The top section contained two samples, the middle section contained one sample, and the bottom section contained two samples. The vertical orientation is broken up on the same manner.
2. For each filter, the average of each of the observed CFU values for each section was divided by the total observed CFU average for the entire filter. This value was then multiplied by 100%. The displayed percentages are the average, standard deviation, and range values for all five pleated filters.

Appendix B

Air Sampling Data for Outpatient, Residential, Administrative, and Research Facilities

Table B.1 Outpatient Facility Single Stage Impactor Sampling Data

Sampling Date	Sampling Period (minutes)	Sampling Location (area)	cfu-1 (plate)	cfu-2 (plate)	cfu/m^3-1	cfu/m^3-2
2/4/2004	5	1	1	3	7	21
2/9/2004	5	1	6	9	43	64
2/16/2004	8	1	13	6	59	27
2/19/2004	15	1	56	91	143	244
3/1/2004	15	1	14	13	34	31
3/3/2004	15	1	24	16	59	39
3/5/2004	15	1	24	38	59	94
3/9/2004	15	1	13	13	31	31
3/11/2004	15	1	12	9	29	22
3/18/2004	15	1	43	55	108	140
3/24/2004	15	1	28	27	69	66
3/29/2004	15	1	95	92	256	247
3/31/2004	15	1	45	39	113	97
4/6/2004	15	1	59	54	151	138
4/8/2004	15	1	13	17	31	41
4/12/2004	15	1	12	21	29	51
4/15/2004	15	1	51	63	129	162
2/4/2004	5	2	5	5	36	36
2/9/2004	5	2	2	3	14	21
2/16/2004	8	2	5	7	22	31
2/19/2004	15	2	30	60	74	154
3/1/2004	15	2	17	13	41	31
3/3/2004	15	2	13	18	31	44
3/5/2004	15	2	30	34	74	84
3/9/2004	15	2	9	9	22	22
3/11/2004	15	2	12	14	29	34
3/18/2004	15	2	40	56	100	143
3/24/2004	15	2	19	14	46	34
3/29/2004	15	2	27	37	66	92
3/31/2004	15	2	22	19	54	46

Sampling Date	Sampling Period (minutes)	Sampling Location (area)	cfu-1 (plate)	cfu-2 (plate)	cfu/m^3-1	cfu/m^3-2
4/6/2004	15	2	12	11	29	27
4/8/2004	15	2	9	12	22	29
4/12/2004	15	2	6	16	14	39
4/15/2004	15	2	10	10	24	24
2/4/2004	5	3	3	3	21	21
2/9/2004	5	3	1	4	7	29
2/16/2004	8	3	8	8	36	36
2/19/2004	15	3	32	18	79	44
3/1/2004	15	3	15	15	36	36
3/3/2004	15	3	12	11	29	27
3/5/2004	15	3	59	74	151	193
3/9/2004	15	3	6	10	14	24
3/11/2004	15	3	4	8	10	19
3/18/2004	15	3	30	46	74	116
3/24/2004	15	3	19	14	46	34
3/29/2004	15	3	27	30	66	74
3/31/2004	15	3	25	21	61	51
4/6/2004	15	3	18	12	44	29
4/8/2004	15	3	12	8	29	19
4/12/2004	15	3	12	9	29	22
4/15/2004	15	3	19	27	46	66
2/4/2004	5	4	0	0	0	0
2/9/2004	5	4	1	3	7	21
2/16/2004	8	4	5	7	22	31
2/19/2004	15	4	23	25	56	61
3/1/2004	15	4	19	14	46	34
3/3/2004	15	4	14	10	34	24
3/5/2004	15	4	17	14	41	34
3/9/2004	15	4	11	14	27	34
3/11/2004	15	4	12	10	29	24
3/18/2004	15	4	29	24	71	59
3/24/2004	15	4	10	8	24	19
3/29/2004	15	4	22	25	54	61
3/31/2004	15	4	15	12	36	29
4/6/2004	15	4	10	12	24	29
4/8/2004	15	4	9	8	22	19
4/12/2004	15	4	11	9	26	22
4/15/2004	15	4	12	11	29	27
2/4/2004	5	5	3	2	21	14
2/9/2004	5	5	4	6	29	43
2/16/2004	8	5	13	6	59	27
2/19/2004	15	5	103	116	281	322
3/1/2004	15	5	11	16	27	39
3/3/2004	15	5	7	7	17	17
3/5/2004	15	5	20	14	49	34
3/9/2004	15	5	6	5	14	12
3/11/2004	15	5	8	8	19	19

Sampling Date	Sampling Period (minutes)	Sampling Location (area)	cfu-1 (plate)	cfu-2 (plate)	cfu/m^3-1	cfu/m^3-2
3/18/2004	15	5	16	15	39	36
3/24/2004	15	5	44	52	110	132
3/29/2004	15	5	40	47	100	118
3/31/2004	15	5	35	27	87	66
4/6/2004	15	5	21	15	51	36
4/8/2004	15	5	13	13	31	31
4/12/2004	15	5	18	17	44	41
4/15/2004	15	5	8	6	19	14
2/4/2004	5	OUT	6	4	43	29
2/9/2004	5	OUT	8	3	58	21
2/16/2004	8	OUT	9	12	41	54
2/19/2004	15	OUT	64	78	165	205
3/1/2004	15	OUT	117	119	326	332
3/3/2004	15	OUT	47	58	118	148
3/5/2004	15	OUT	112	95	309	256
3/9/2004	15	OUT	31	29	77	71
3/11/2004	15	OUT	42	36	105	89
3/18/2004	15	OUT	200	200	650	650
3/24/2004	15	OUT	70	54	182	137
3/29/2004	10	OUT	153	152	679	673
3/31/2004	10	OUT	124	136	524	586
4/6/2004	10	OUT	59	54	226	206
4/8/2004	10	OUT	28	27	103	99
4/12/2004	10	OUT	21	22	77	81
4/15/2004	10	OUT	51	63	194	243

In Table B.2 sampling location column codes are as follows:

1=home 1 outdoor,

2=home 1 first floor,

3=home 1 basement,

4=home 2 outdoor,

5=home 2 first floor,

6=home 2 basement,

7=home 3 outdoor,

8=home 3 first floor,

9=home 3 basement.

Table B.2 Three Home Single Stage Impactor Sampling Data

Sampling Date	Sampling Period (minutes)	Sampling location	cfu-1 (plate)	cfu-2 (plate)	cfu/m^3-1	cfu/m^3-2
5/25/2004	5	1	157	144	1403	1258
5/26/2004	5	1	116	132	967	1129
5/27/2004	5	1	132	135	1129	1161
5/28/2004	5	1	97	93	785	749
5/25/2004	5	1	165	172	1497	1582
5/26/2004	5	1	60	85	461	676
5/27/2004	5	1	255	230	2853	2404
5/28/2004	5	1	74	46	580	347
5/25/2004	10	2	46	102	174	416
5/26/2004	10	2	111	115	459	479
5/27/2004	10	2	111	106	459	435
5/28/2004	10	2	127	120	539	503
5/25/2004	10	2	85	62	338	239
5/26/2004	10	2	83	82	329	325
5/27/2004	10	2	143	125	623	529
5/28/2004	10	2	65	53	251	202
5/25/2004	10	3	106	105	435	430
5/26/2004	10	3	179	175	834	809
5/27/2004	10	3	120	110	503	454
5/28/2004	10	3	98	98	397	397
5/25/2004	10	3	52	60	198	231
5/26/2004	10	3	59	62	226	239
5/27/2004	10	3	74	68	290	264
5/28/2004	10	3	64	61	247	235
5/25/2004	5	4	133	131	1140	1119
5/26/2004	5	4	152	128	1346	1088
5/27/2004	5	4	169	145	1545	1269
5/28/2004	5	4	74	82	580	650
5/25/2004	5	4	156	105	1392	860
5/26/2004	5	4	91	96	730	776
5/28/2004	5	4	86	70	685	545
5/25/2004	10	5	57	52	218	198
5/26/2004	10	5	29	23	107	84
5/27/2004	10	5	58	72	222	281
5/28/2004	10	5	17	25	62	92
5/25/2004	10	5	42	35	158	130
5/26/2004	10	5	22	23	81	84
5/28/2004	10	5	46	50	174	190
5/25/2004	10	6	130	115	554	478
5/26/2004	10	6	136	130	586	554
5/27/2004	10	6	57	59	218	226
5/28/2004	10	6	79	83	312	329
5/25/2004	10	6	93	93	374	374

Sampling Date	Sampling Period (minutes)	Sampling location	cfu-1 (plate)	cfu-2 (plate)	cfu/m^3-1	cfu/m^3-2
5/26/2004	10	6	45	26	170	96
5/28/2004	10	6	81	74	320	290
5/25/2004	5	7	96	113	776	938
5/26/2004	5	7	118	104	987	851
5/27/2004	5	7	135	129	1161	1098
5/28/2004	5	7	107	127	880	1078
5/26/2004	5	7	70	65	545	503
5/27/2004	5	7	148	137	1302	1182
5/28/2004	5	7	64	54	494	412
5/25/2004	10	8	201	155	981	690
5/26/2004	10	8	41	57	154	218
5/27/2004	10	8	82	71	325	277
5/28/2004	10	8	26	27	96	99
5/26/2004	10	8	94	91	379	365
5/27/2004	10	8	40	60	150	231
5/28/2004	10	8	400	400	6098	6098
5/25/2004	5	9	400	400	12196	12196
5/26/2004	5	9	111	113	918	938
5/27/2004	5	9	146	127	1280	1078
5/28/2004	5	9	106	110	870	908
5/26/2004	5	9	224	223	2307	2291
5/27/2004	5	9	146	110	1280	908
5/28/2004	5	9	292	243	3690	2628

Table B.3 Administrative Facility Single Stage Impactor Sampling Data

Sampling Date	Sampling Period (minutes)	Sampling Location (area)	cfu-1 (plate)	cfu-2 (plate	cfu/m^3-1	cfu/m^3-2
3/10/04	15	1	17	15	41	36
3/12/04	15	1	26	33	64	82
3/19/04	15	1	25	13	61	32
3/24/04	15	1	8	12	19	29
3/30/04	15	1	12	23	29	56
3/31/04	15	1	27	34	66	84
4/8/04	15	1	22	19	54	46
4/13/04	15	1	16	15	39	36
4/16/04	15	1	15	12	36	29
4/20/04	15	1	16	18	39	44
3/10/04	15	2	5	5	12	12
3/12/04	15	2	9	7	22	17
3/19/04	15	2	14	21	34	51
3/24/04	15	2	6	9	14	22
3/30/04	15	2	19	12	46	29
3/31/04	15	2	30	29	74	34
4/8/04	15	2	14	14	34	34
4/13/04	15	2	14	23	34	56
4/16/04	15	2	10	15	24	36
4/20/04	15	2	12	12	29	29
3/31/04	10	out	135	129	581	549
4/8/04	10	out	185	191	873	913
4/13/04	10	out	39	20	146	73
4/16/04	10	out	120	97	504	393
4/20/04	10	out	175	162	809	731

Table B.4 Research Facility Single Stage Impactor Sampling Data

Sampling Date	Sampling Period (minutes)	Sampling location	cfu-1 (plate)	cfu-2 (plate)	cfu/m^3-1	cfu/m^3-2
3/9/2004	15	East	11	5	27	12
3/11/2004	15	East	6	4	14	10
3/19/2004	15	East	13	18	31	44
3/23/2004	15	East	10	15	24	36
3/29/2004	15	East	10	9	24	22
3/31/2004	15	East	6	9	14	22
4/8/2004	15	East	5	4	12	10
4/12/2004	15	East	6	5	14	12
4/16/2004	15	East	5	6	12	14
4/20/2004	15	East	11	9	27	22
3/9/2004	15	West	8	7	19	17
3/11/2004	15	West	2	2	5	5
3/19/2004	15	West	27	32	66	79
3/23/2004	15	West	28	27	69	66
3/29/2004	15	West	15	12	36	29
3/31/2004	15	West	9	13	22	31
4/8/2004	15	West	7	11	17	27
4/12/2004	15	West	13	5	31	12
4/16/2004	15	West	6	10	14	24
4/20/2004	15	West	12	16	29	39
3/11/2004	15	out	35	29	87	71
3/19/2004	15	out	64	60	165	154
3/23/2004	15	out	47	60	118	154
3/29/2004	15	out	134	141	383	408
3/31/2004	10	out	58	54	222	206
4/8/2004	10	out	26	21	96	77
4/12/2004	10	out	13	18	47	66
4/16/2004	10	out	59	55	226	210
4/20/2004	10	out	56	52	214	198

VITA

VITA

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