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HVAC FILTERS AS A SAMPLING MECHANISM FOR INDOOR CONTAMINANTS

Committee:

_____

Kerry Kinney, Supervisor

_____

Jeffrey Siegel, Co-Supervisor

_____

Richard Corsi

_____

Mary Jo Kirisits

_____

Paul Szaniszlo

HVAC FILTERS AS A SAMPLING MECHANISM FOR INDOOR CONTAMINANTS

by

Federico Noris, B.S.; M.S.

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Dedication

To my mom, dad, sister and Val

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HVAC FILTERS AS A SAMPLING MECHANISM FOR INDOOR CONTAMINANTS

Federico Noris, Ph.D.

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Supervisor(s): Kerry Kinney and Jeffrey Siegel

Indoor air quality investigations often focus on air and settled dust samples to assess chemical and biological contamination. Although the information provided by these techniques is useful, HVAC filters represent a new option for investigating contaminants in the indoor environment. This dissertation explores the potential use of HVAC filters as long-term, passive samplers by investigating the contaminants found in HVAC dust and other indoor locations and by evaluating the likelihood that HVAC filters will capture indoor particles. A field investigation of heavy metal and culturable microbial contaminants found in air, settled dust and HVAC filter dust corroborated the hypothesis that HVAC filters hold promise as a sampling mechanism in residences. However, several factors including filter efficiency, HVAC cycling and particle size seemed to influence the results. Also, it was unclear how the composition of the microbial communities varied with sampling location. Subsequently, the bacterial and fungal communities present in several sampling locations within residences and in an unoccupied test house were investigated. In residences, the microbial communities encountered in HVAC filter dust were not different from those in high surface dust. High efficiency HVAC filters also seem to be a viable alternative to long-term air sampling. Occupants influence the composition of the microbial communities in residences and are

associated with Actinobacteria and Firmicutes, while Proteobacteria dominate the air samples and might have an outdoor air origin. A fate analysis to assess the magnitude of the different particle removal mechanisms revealed that small and large particles are likely to deposit on surfaces, while intermediate sized particles stay suspended in air longer. HVAC filters can collect particulate matter over a broad size range and may be effective overall samplers of particle-bound contaminants. Nevertheless, filter efficiency and air recirculation rate are important parameters that influence the likelihood that filters will capture particles, while air exchange rate has little effect. The results from this study indicate that HVAC filters can be used as an alternative to traditional indoor sampling mechanisms for contaminants associated with particles.

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1. INTRODUCTION

1.1 PROBLEM STATEMENT

People spend the majority of their time indoors and their overall exposure to pollutants is dominated by indoor contaminant levels (USEPA, 2004). In order to estimate the exposure of building occupants, the concentrations of contaminants present indoors have been intensively studied, typically by collecting air and settled dust samples in various indoor locations (Adgate *et al.*, 1998; Bouillard *et al.*, 2005; Ross *et al.*, 2000). Although useful as a characterization technique, the specific contaminants and concentrations found in particles suspended in air and in settled dust are often different (Rudel *et al.*, 2003) possibly due to the fact that air and settled dust sampling methods preferentially sample different particle size ranges. Additionally, both types of sampling locations may have pitfalls and limitations including the spatial and temporal variability of the samples collected (Douwes *et al.*, 2003; Skov *et al.*, 1990). Air sampling techniques are usually short in duration and are influenced by the specific location where the samples are collected. Therefore, air samples can be considered a snapshot of a particular contaminant at that time and place. Dust samples may represent more integrated (longer-term) samples, but may have other issues. Settled dust may overemphasize larger particles that are more likely to settle by gravity. Floor dust is influenced by tracked-in dust that may not be representative of dust of indoor origin. Additionally, the contaminant concentrations in dust samples can be normalized by the surface area sampled or by the mass of particles collected, which leads to ambiguity when comparing values in the literature.

An alternative sampling approach that has received little attention is the use of heating ventilation, and air-conditioning (HVAC) filters as a sampling mechanism. More than 70% of US homes have a central air conditioning system (US Bureau of Census,

2005), almost all with a built-in filtration system making these filters widely available. These filters are in place for extended periods of time and, during their lifetime, capture a significant amount of particles. Thus, they may serve as long-term, passive samplers that can be collected with minimal effort and analyzed for a wide range of indoor contaminants.

1.2 OBJECTIVES

The present research focused on the evaluation of filters as a sampling mechanism by investigating: (1) biological and heavy metal concentrations in residences, (2) microbial community composition in residences and in a full-scale test house, and (3) the fate of indoor airborne particles. More specifically, the objectives of this study were to:

- Compare the concentrations of culturable microorganisms and heavy metals present in HVAC filter dust to those found in settled dust and air samples.
- Compare the HVAC filter microbial communities to those of surface dust and air in residences and in a full-scale test house.
- Investigate the influence of occupants and the contribution of outdoor air to the development of indoor microbial communities.
- Evaluate potential of filter samplers by analyzing the fate of indoor airborne particles and the importance of removal mechanisms for typical residential scenarios.
- Identify and investigate the role of critical parameters that may affect the use of HVAC filters as passive samplers of indoor particles.

1.3 SCOPE

This investigation was divided into three major phases:

- Phase I: Culturable microorganisms and heavy metals. A field investigation was conducted to assess the concentration of contaminants in floor, high surface and HVAC filter dust as well as in indoor air at eight residences.
- Phase II: Microbial communities. A field investigation was implemented to determine the composition of the bacterial and fungal communities present in high surface and HVAC filter dust samples in four residences and in a full-scale test house. For comparison, the microbial communities in indoor and outdoor air were also characterized at the test house.
- Phase III: Fate analysis of indoor airborne particles. A modeling approach was utilized to predict the fate of indoor particles for a range of scenarios. Experimental validation of the model was conducted at a full-scale test house under controlled conditions.

The first phase focused on comparing the concentrations of culturable microorganisms and heavy metals present in settled and HVAC filter dust samples in order to explore the use of filters as samplers and to identify key parameters that merited further investigation in the following phases. During the second phase, we expanded the study to a culture-independent, DNA-based approach capable of revealing a much larger range of microorganisms. The goal of this phase was to compare the composition of the microbial communities to evaluate the use of filters as samplers for microbial contamination and to identify any associations of specific microbial groups with sampling location and occupants. Finally, in the third phase, we applied and validated a model to predict the fate of indoor airborne particles and to assess the likelihood of particle capture in a HVAC filter as a function of particle size, filter efficiency, air recirculation rate, and air exchange rate.

1.4 ORGANIZATION

This dissertation is divided into two major parts. The first part is an executive summary that includes a literature review, an overview of the methodology, a summary of the results, and overall conclusions. This material follows the three phases presented in Section 1.3. The second part of the dissertation consists of Appendices A, B and C that contain the complete text of the supporting papers described in the three research phases. These papers are referenced throughout the dissertation and are listed below:

- Appendix A: Noris, F., Siegel, J.A., Kinney, K.A. 2009. Biological and metal contaminants in HVAC filter dust. ASHRAE Transactions, 115, part 2, 484-491
- Appendix B: Noris F., Siegel, J.A, Kinney, K.A. Evaluation of HVAC filters as a sampling mechanism for indoor microbial communities (in preparation).
- Appendix C: Noris, F., Kinney K.A., Siegel J.A. Fate analysis of indoor particles and evaluation of HVAC filters as samplers (in preparation)

In addition, supplemental information related to the DNA-based techniques employed in the research as well as the metrics utilized to analyze the phylogenetic data is provided in Appendices D and E.

2. BACKGROUND

This section presents a brief review of the literature relevant to the use of HVAC filters as a sampling mechanism for indoor contaminants.

2.1 CULTURABLE MICROORGANISMS AND HEAVY METALS

Indoor air quality researchers have studied biological, chemical and particulate contamination in indoor environments, the health effects and discomfort that these contaminants can cause, and their removal from indoor air and surfaces. Two important broad categories of contaminants of concern are microorganisms and heavy metals. The presence of microorganisms indoors has been associated with health issues and discomfort including respiratory problems and occupant dissatisfaction (Gyntelberg *et al.*, 1994; Verhoeff and Burge, 1997). Exposure to toxic heavy metals can cause damage to the central nervous system, the liver and bones (Moore, 1990).

The reported bacterial and fungal concentrations in air range from 10^2 to 10^4 colony forming units (CFU)/m^3 while typical settled dust concentrations are on the order of 10^5-10^7 CFU/g, (Bouillard *et al.*, 2005; Dales *et al.*, 1997; Gorny and Dutkiewicz, 2002; Nilsson *et al.*, 2004; Ross *et al.*, 2000). However, the literature values are difficult to compare because they vary substantially depending on sampling technique, building use, and sampling location among other factors. Some have associated indoor microbial concentrations, mainly molds, to asthma symptoms (Bjornsson *et al.*, 1995; Park *et al.*, 2006; Ross *et al.*, 2000; Smedje *et al.*, 1997). However, the association between fungal culturable concentrations and respiratory problems has been inconsistent, suggesting that we are not assessing human exposure to microorganisms correctly.

Several researchers have investigated particle composition in terms of organic and inorganic compounds (Adgate *et al.*, 1998; Stranger *et al.*, 2007; Wang *et al.*, 2006).

Metal concentrations in house dust are generally in the μg g^{-1} range, except for Pb and Zn which are generally in the mg g^{-1} range (Adgate *et al.*, 1998; Oliver *et al.*, 1999). The correlation with potential indoor and outdoor sources as well as particle size distributions have also been investigated (Al-Rajhi *et al.*, 1996; Chattopadhyay *et al.*, 2003; Decker *et al.*, 2002; Kim *et al.*, 1998; Tong, 1998). Wang *et al.* (2006) reported a strong correlation between indoor particulate matter (PM) and indoor metal concentrations. This correlation was stronger than that observed outdoors, suggesting indoor sources of metals. They also reported a higher mean metal concentration in PM_{10} (PM < 10 μm in size) than in $PM_{2.5}$ (PM <2.5 μm), even though several critical elements (Pb, Cr, Cd and As) were present at higher concentrations in the smaller size fraction. Other studies have suggested a correlation between fine particles and health problems (Berico *et al.* 1997; Heidi, 2000).

The inconsistent association between contaminant concentrations and health symptoms may be attributable to some of the limitations in exposure assessments including the spatial and temporal variability of the samples (Douwes *et al.*, 2003; Skov *et al.*, 1990) and the reliance on culture-based methods for microbial contaminants. Air samples are typically short-term in nature and provide only a snapshot of contaminants in air at a particular time and place. Even when collected from the same location, airborne bacterial samples have significant temporal variability (Fierer *et al.*, 2008), highlighting the need to develop an integrative methodology to assess biological contaminants. Floor dust provides an integrated sample of contaminants but these samples are influenced by material tracked-in from the outside and may be skewed toward large particle-bound contaminants. Nevertheless, settled dust has been used extensively in indoor investigations (Koch *et al.*, 2000; Rintala *et al.*, 2008; Tong, 1998). Recently, Stanley *et al.* (2008) utilized filters as bioaerosol sampling devices in two large public buildings to

determine the culturable concentrations of selected bacteria present in air. The author is not aware of any researchers who have utilized HVAC filters as samplers to characterize indoor metal concentrations.

2.2 INDOOR MICROBIAL COMMUNITIES

All of the biological studies reported in Section 2.1 relied on traditional culturing methods for the quantification and detection of microorganisms. These methods are selective since no culture medium is suitable for the growth of all microbes. Toivola *et al.* (2002) estimated that less than 1% of the microorganisms present indoors are culturable. The lack of a broad characterization of the microbial communities could lead to an incomplete assessment of human exposure and potentially could be responsible for the inconsistent association between microbial contamination and respiratory symptoms.

In recent years, molecular-based tools have been developed that offer the promise of being able to detect a much greater fraction of the microbial community, not just the culturable fraction. Several recent studies have applied culture-independent, DNA-based approaches, to better characterize the diverse bacterial and fungal communities present in indoor environments (Kelley *et al.* 2004; Pakarinen *et al.*, 2008; Pitkäranta *et al.*, 2008; Rintala *et al.*, 2008; Täubel *et al.*, 2009; Tringe *et al.*, 2008). The application of molecular biology tools to indoor environmental investigations should reveal a much greater fraction of the microbial community present than does culturable methods, a finding recently confirmed by Pitkäranta *et al.* (2008). They compared the fungal communities in two office buildings over four seasons using culture-based and molecular-based techniques; the microbial community identified in the buildings by culture-based techniques differed considerably from that identified using the molecular-based techniques. Vesper *et al.* (2007) reported an association between asthma

symptoms and the Relative Moldiness Index (RMI), an index based on molecular biology tools, suggesting that these techniques might provide a better characterization of human exposure to microorganisms. While most of the molecular-based studies described above focused mainly on settled dust, Tringe *et al.* (2008) investigated the bacterial communities present on the dust that collected on two HVAC filters in two large shopping centers in Singapore. They reported that the microbial communities present in the two HVAC filters (which they regarded as representative of the community present in the indoor air) had more in common with each other than with the other environmental samples collected nearby. They also found greater similarity between the bacterial communities in filter samples and indoor floor dust compared to those present in outdoor ground-level dust suggesting that the filter community originates from an indoor niche.

2.3 FATE OF INDOOR PARTICLES

Particle size may influence the fate of indoor particles and, as a consequence, different sampling locations may preferentially oversample some particle size ranges. Specifically, settled dust may be biased toward larger particles that are more likely to deposit by gravity onto surfaces. In contrast, air samples may tend to preferentially collect particles with sizes that are not effectively removed by other mechanisms such as deposition and filtration. In order to compare the contaminant concentrations observed in samples collected from various locations, the fate of indoor airborne particles and their likelihood to be removed by the different mechanisms needs to be investigated.

The fate of indoor airborne particles is a complex phenomenon with several competing mechanisms that are influenced by a variety of parameters, including the specific characteristics of the building and of the HVAC filtration system as well as by the particle size of interest. The main indoor particle removal mechanisms are deposition

onto surfaces, exfiltration through the building envelope and, if the HVAC system is being operated, HVAC filtration. Particle deposition onto indoor surfaces as a function of particle size has been widely studied (Long *et al.* 2001; Riley *et al.*, 2002; Thatcher and Layton, 1995) mostly in controlled laboratory chambers. Riley *et al.* (2002) reported that loss processes vary with building conditions and operation and are strongly particle-size dependent. Air exchange rate and exfiltration of particles affect indoor particle concentrations as reported by Abt *et al.* (2000). HVAC filters are capable of removing indoor airborne particles (Hanley *et al.*, 1994) and play a critical role in the decay of particle concentration in indoor environments (Fisk *et al.*, 2000). Wallace *et al.* (2004) investigated the impact of a central fan and mechanical filters and reported that filters can effectively reduce indoor air particle concentrations with increased removal rates by up to 2 h^{-1} for fine and ultrafine particles. Siegel and Waring (2008) observed the influence of HVAC filter efficiency, time of operation and particle size on the loading rates of HVAC filters. Zhao and Wu (2009) investigated particle fate in ventilation systems, including filters, for a range of different scenarios and reported a strong dependency on particle size. To evaluate the merits of utilizing HVAC filters as passive samplers, the current study expands on this particle fate analysis to assess the likelihood of particle capture on filters for a range of building and HVAC scenarios.

3. METHODS

A summary of the experimental and modeling approaches adopted for the current investigation are presented in this section. An overview of the experimental approach is provided below and additional details regarding the methodology are available in Appendices A, B, C, and D.

3.1 EXPERIMENTAL APPROACH

In Phase I of this dissertation, the microbial and metal concentrations in floor, surface and HVAC filter dust in addition to air in eight residences in Austin, Texas, are compared. These investigation provided an exploration of the relationships between contaminant concentrations observed in different indoor locations and the importance of a variety of factors such as filter efficiency, air recirculation rate and particle size.

Phase II of the dissertation focused on the characterization of the bacterial and fungal communities on HVAC filters using a culture independent technique. The microbial communities that develop on HVAC filters were compared to those present in indoor settled dust and in air within residences and in a mostly unoccupied test house. This phase expanded upon the Phase I investigation which focused only on the culturable microbial concentrations present in samples collected from different indoor locations. Additionally, the association between microbial groups and specific sampling locations as well as the influence of occupants was evaluated. The findings from this investigation integrate the current knowledge regarding the use of HVAC filters as a sampling mechanism for microbial contaminants (Stanley *et al.*, 2008; Tringe *et al.*, 2008) and the association between specific bacterial groups and occupants (Rintala *et al.*, 2008; Täubel *et al.*, 2009).

In Phase III, a modeling approach capable of predicting the removal probabilities of indoor particles by different mechanisms was validated and then applied to typical residential scenarios. The influence of filter efficiency, air recirculation rate, and air exchange rate on the size-dependent particle fate was assessed. The likelihood of HVAC filters to collect particles was evaluated in order to delineate the conditions and particle sizes for which HVAC filters are most likely to be effective samplers. Additionally, I wanted to compare the use of HVAC filters to more traditional sampling approaches such as periodic air measurements or settled dust collection. The results from this analysis will be useful for assessing the effectiveness of using HVAC filters as an indoor sampling technique.

3.2 CULTURABLE MICROBIAL CONTAMINANTS AND HEAVY METALS

A sample of convenience of eight residences and one commercial building located in Austin, Texas was selected for this investigation. Floor dust, high surface dust and HVAC filter dust samples were collected 2-3 times over a six-month period in each residence during the cooling season (summer and fall), while for the commercial site, only HVAC filter samples were collected. During the cooling season, HVAC systems are used more frequently and thus provide more ideal conditions for testing the validity of utilizing filters as a sampling mechanism. Two HVAC filters were collected from the sites approximately three months apart, while the settled dust samples were collected approximately four weeks apart. For a subset of five buildings, floor and high surface dust samples as well as indoor air samples were collected on three separate occasions.

The enumeration of culturable bacteria and fungi present in the bioaerosol samples, settled dust, and HVAC dust samples was completed using the standard spread-plate method 9215C (APHA, 1998). To estimate the spore-forming fraction of the

population, an aliquot of each sample was pasteurized for 15 minutes at 75º C, following the procedure developed by Barbeau *et al.* (1997), and then plated as described above. Negative controls and blanks were analyzed as well. Heavy metal concentrations in the HVAC filter, floor, and high surface dust were determined via atomic absorption spectroscopy (Perkin Elmer AAnalyst 600). Dust samples were digested according to the microwave-assisted digestion method 3030K (APHA, 1998) and the liquid extract from each sample was analyzed for selected heavy metals (Pb, As, Cd) according to method 3111B (APHA, 1998). A nonparametric statistical method, the Wilcoxon Rank-Sum Test, which does not assume any specific distribution of the data, was applied to compare and identify dissimilarities among the different data groups. A significance level of 0.1 was assumed owing to the small sample size and the conservative nature of this statistical test.

3.3 MICROBIAL COMMUNITY INVESTIGATION

In the second phase of the investigation, the analysis was expanded to a culture-independent approach potentially capable of more fully characterizing the microbial communities present indoors. Additional information regarding the methodology employed can be found in Appendices B and D. This phase was conducted in a subset of four of the above residences and in an unoccupied 110 m^2 manufactured home (test house) where the fan of the HVAC system was operated continuously during the investigation. The test house was mainly unoccupied, which reduced localized particle and microbial emissions and represented a good site to conduct detailed measurements. High-efficiency (minimum efficiency reporting values, MERV > 11) polyester HVAC filters were installed in all of the sites at the beginning of this phase and, at filter

installation, several high surfaces were cleaned and the homeowners were instructed not to clean the designated surfaces.

Filters and high surface samples were collected two months after the installation of the filter in the occupied residences and one month after installation in the test house. This difference was due to the fact that in test house the HVAC system operated continuously and thus collected particles continuously. In the residences, the occupants had control over the HVAC system and, as a result, the systems operated only a fraction of time. As a result, the HVAC system in the test house operated a greater time and filtered a greater volume of air than the systems in the residences; however, the particle concentrations in the test house were expected to be lower so the additional filtering time was necessary to collect sufficient particles for analysis. At the time of filter removal in the residences and in the test house, a composite sample of high surface dust from the previously cleaned surfaces was collected using a vacuum mechanism. During the month-long investigation in the test house, indoor and outdoor bioaerosol samples were collected 5 days per week using an impinger method. The microorganisms present in each sample collected from the residences and the test house were transferred to a 0.2-µm GTTP Membrane Filter (Millipore, Billerica, MA) by filtration. The DNA from the microorganisms captured on the 0.2-µm filters was extracted using a modified version of the Power Soil DNA (MoBio Laboratories, Carlsbad, CA) kit and amplified through PCR reactions with primers 8F (5'-AGAGTTTGATCCTTGGCTCAG-3') and 1492R (5'-GCYTACCTTGTTACGACTT-3') for bacterial DNA amplification or fungal-specific primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). These primers have been used successfully in several other microbial community studies (O'Brien *et al.*, 2005) and are useful for delineating and comparing the fungal community present in the samples collected from

different indoor locations. However, as with all molecular tools, it is acknowledged that a different set of primers (e.g., EF4 and fung5 or fun18Sf and ITS4) may lead to the amplification of different microbial species (Lauber *et al.,* 2008; Pitkäranta *et al.,* 2008) and the results of any microbial community analysis must be interpreted with caution. Following PCR amplification, the amplicons were cloned using the TOPO TA cloning kit for sequencing (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions and subsequently sequenced in one direction with an ABI 3730 DNA analyzer (Applied Biosystems, Foster City, CA). The nonredundant sequences from the current study were deposited in the GenBank database with accession numbers GU595461-GU596375 for the bacterial clones and GU721174-GU722092 for the fungal clones.

Sequences were aligned against the GenBank database utilizing the BLAST algorithm (Altschul *et al.*, 1990) using 97% sequence similarity as the criterion to determine the similarity to known microorganisms. The web-based tool FastGroupII (Yu *et al.*, 2006) was used to estimate the number of Operational Taxonomic Units (OTUs), Chao1 richness estimator, the Shannon-Wiener diversity index, and to perform the rarefaction analysis. The Chao1 (Chao, 1994) is a predictor of the minimum richness that is based on the rare ribotypes within a sample. The Shannon-Wiener index estimates the diversity by taking into account the number of ribotypes and their abundance. The rarefaction analysis (Appendix E) estimates the total richness of the sample and the influence that a smaller sample size may have on the ribotypes identified. The information provided by these parameters was used to assess the diversity of the communities as well as the representativeness of the samples collected relative to the predicted community composition. The sequences amplified from bacteria and fungi were then aligned separately using MEGA (Tamura *et al.*, 2007). Phylogenetic trees were created in the CIPRESS portal (http://www.phylo.org/portal) using the RAxML

algorithm (Stamatakis *et al.*, 2005). Finally, the microbial communities present in the different samples were compared using the Weighted UniFrac algorithm (Lozupone *et al.*, 2006; Lozupone *et al.*, 2007). A significance level of 0.1 was assumed due to the reduced number of sites and the exploratory nature of the investigation. More details on these metrics and their limitations appear in Appendix E.

3.4 FATE ANALYSIS

The objective of this phase of the dissertation was to investigate the fate of indoor airborne particles and the likelihood that HVAC filters can be effective samplers for indoor particle-bound contaminants. A scaling analysis was performed to estimate the probability that 0.001-100 μm particles would be removed from indoor air in a typical residence by deposition, exfiltration, or filtration through the HVAC filter. The volume of the residence was selected considering a typical floor area of 163.3 m^2 (US Bureau of Census, 2005) and assuming a ceiling height of 2.4 m for a total volume (V_T) of 391.9 m^3. To estimate the removal probability for each mechanism, the size-dependent characteristic time was considered for each removal process. The characteristic time for deposition was the particle size-resolved deposition loss rate coefficients (β), for exfiltration, the air exchange rate (λ) was utilized, while for filtration, the recirculation rate (λ_r) multiplied by the size-dependent filter removal efficiency (η) was used. The air exchange rate is the ratio between the flow rate in and out of a building (Q) and the volume of the residences, while the air recirculation rate is the ratio between the airflow through the HVAC system (Q_r) and the volume of the residences.

For deposition, the β values summarized by Riley *et al.* (2002) were utilized in the model. For exfiltration, the 10^{th}, 50^{th} and 90^{th} percentile values of the λ distribution reported by Murray and Burmaster (1995) were utilized. These values, corresponding to

λ = 0.2, 0.5 and 1.3 h^{-1}, were used to evaluate how the tightness of the residence may affect the potential use of HVAC filters as passive samplers. For the loss rate due to filtration, the λ_r multiplied by the size-dependent removal efficiency (η) of the HVAC filter was utilized. Two different λ_r values (5.2, 1.1 h^{-1}) were considered by assuming either continuous operation for mechanical ventilation (λ_r= 5.2 h^{-1}) or cyclic duty operation (λ_r= 1.1 h^{-1}) for a typical 3-ton air conditioner operating 22% of the time (Appendix A). Three different clean filters with (MERV), as determined by ASHRAE Standard 52.2 (ASHRAE, 2007), of <5, 6 and 11 were considered using the filtration efficiencies employed by Waring and Siegel (2008). For each scenario, *j*, considered, the size-dependent characteristic times for each mechanism were then normalized by the sum of all the characteristic times, k_j, and the resulting fraction represented the relative removal probability of that mechanism for a given particle size. The sum of the characteristic times, k_j, was calculated as follows:

$$k_j = \beta + \lambda_j + \lambda_{r,j}\eta_j \qquad (1)$$

As a consequence, for a particular scenario, the size-dependent particle removal probability via each mechanism, $p_{r,m}$, was estimated with the model as follows:

$$p_{r,m} = \frac{r_{j,m}}{k_j} \qquad (2)$$

where $r_{j,m}$ is the size-dependent characteristic time of each process for a particular scenario.

The validation of the modeling approach was performed in a 110 m^2 (volume of 250 m^3) unoccupied test house. Approximately 50 g of Ultrafine Arizona Test dust (Powder Technology, Burnsville, MN) was dispersed into the house using a dust sprayer

and mixing fans. Eight mixing fans and one ceiling fan were operated to improve the mixing of the injected particles. Six Aerotrak Handheld Particle Counters (TSI Inc., Shoreview, MN) were located in different indoor locations: living room, kitchen, upstream and downstream of the HVAC filter and one in each of two bedrooms. We measured particle concentrations in the following size bins: 0.3-0.5, 0.5-1, 1-3, 3-5, 5-7, 7-10 μm. Experiments with high (MERV 12) and low (MERV 2) efficiency filters were conducted in triplicate. Each experiment lasted approximately 120 min. To estimate the mass accumulated on each filter, the filters were weighed before and after the experiment using a balance (Sartorius B310S, Goettingen, Germany). Prior to the beginning of the experiments, particle decay tests were performed to estimate the removal of particles due to deposition onto surfaces when the HVAC system was off. For these particle decay tests, approximately 10 g of dust was sprayed and the concentrations were measured as explained below. In this way, the deposition loss coefficient (β) in the test house could be determined for conditions similar to those present during the tests.

At the beginning of each experiment, all surfaces and floors were cleaned and a clean filter was installed. High surface and floor samples were collected at the end of each test using dust collectors. The particle mass deposited on high surfaces and on the floor was measured by weighing the dust collectors before and after the sampling. During the tests, the house was pressurized and the air exchange rate was assessed by the best fit to exponential decay of CO_2 concentrations versus time, correcting for background CO_2. The size-resolved number of particles removed by filtration, n_f, was estimated using the following equation for filtration:

$$n_f = \sum_{t=0}^{t=120} \left(C_{up} - C_{down}\right) Q_r t \qquad (3)$$

where C_{up} and C_{down} are the measured size-resolved concentrations (#/m^3) upstream and downstream of the HVAC filter, Q_r is the flow rate (m^3/h) through the filter, and t is the duration of each experiment (h). The size-resolved n_f was then multiplied by a characteristic volume for each size bin (assuming spherical particles) to obtain the volume of particles collected on the filter, v_f. Using the particle size distribution provided by the manufacturer for the Ultrafine Arizona Test dust, the size-resolved volume of particles injected, v_i, was calculated assuming a constant density across particle size ranges. The ratio between v_f and v_i represents the estimated fraction of filter removal for each size bin during the experiments, $p_{f,e}$:

$$p_{f,e} = \frac{v_f}{v_i} \qquad (4)$$

4. RESULTS AND DISCUSSION

The major findings from the field investigations of contaminants in residences, the experiments in a more controlled full-scale test house and the fate analysis of indoor airborne particles are discussed in this section. More details can be found in Appendices A, B, C, and E

4.1 CULTURABLE MICROBIAL CONTAMINANTS AND HEAVY METALS

Table 1 characterizes the nine sites and the presence of likely sources of contamination. Site 9 was the one light commercial building included in the study. Cooling duty cycles (the fraction of time that the HVAC system operates) at the sites ranged from 9 to 34%. These sites represent the range of HVAC systems and operating characteristics for this region of the country.

Table 1. Site Characteristics

Site #	Year built	# of occupants	Proximity to highway [km (miles)]	Attached garage	Carpet	Filter location	Conditioned Volume [m^3 (ft^3)]	Cooling duty cycle [%]
1	1975	2	1.0 (0.62)	Yes	No	Unit	422 (14,900)	14
2	1973	2	0.6 (0.37)	Yes	Yes	Unit	309 (10,900)	16
3	1998	1	0.2 (0.12)	Yes	No	Register[2]	114 (4,020)	9
4	1998	1	0.2 (0.12)	Yes	Yes	Register	227 (8,010)	27
5	1949	2	1.8 (1.12)	No	No	Register	276 (9,740)	32
6	1941	4	1.1 (0.68)	No	Yes	Register	324 (11,400)	29
7	Late 70s[1]	4	0.6 (0.37)	No	Yes	Unit	259 (9,140)	34
8	1984	3	0.5 (0.31)	Yes	Yes	Unit	308 (10,900)	15
9	1995	3	0.2 (0.12)	No	Yes	Register[3]	656 (23,200)	19

[1]Estimated based on neighborhood and nearby homes.
[2]Three filters in different return grilles were present at this site
[3]Two filters in different return grilles were present at this site

Eighteen HVAC filters, two from each site, were collected and evaluated during the project. As expected, a correlation was observed between filter efficiency and particle mass accumulated on the filter. The mean mass accumulated on the low-efficiency and mid-efficiency filters was 1.7 and 4.0 g, respectively. There also may be a correlation between the mass of particles accumulated on filters and the presence of carpet in the house. The mean mass accumulated on the filters from the sites with and without carpet was 3.9 and 0.8 g, respectively. Carpets tend to accumulate more dust than bare floors because they are harder to clean than other types of floor. As a consequence, particle resuspension from carpet is expected to be greater than from other floor surfaces (Yoon and Brimblecombe, 2000). As demonstrated by Corsi *et al.* (2008), resuspension of PM_{10} is much larger than $PM_{2.5}$, suggesting that even the low MERV filters can retain many of the larger particles from vacuuming activities.

Figure 1 shows the mean bacterial and fungal culturable concentrations for each of the residential sampling locations.

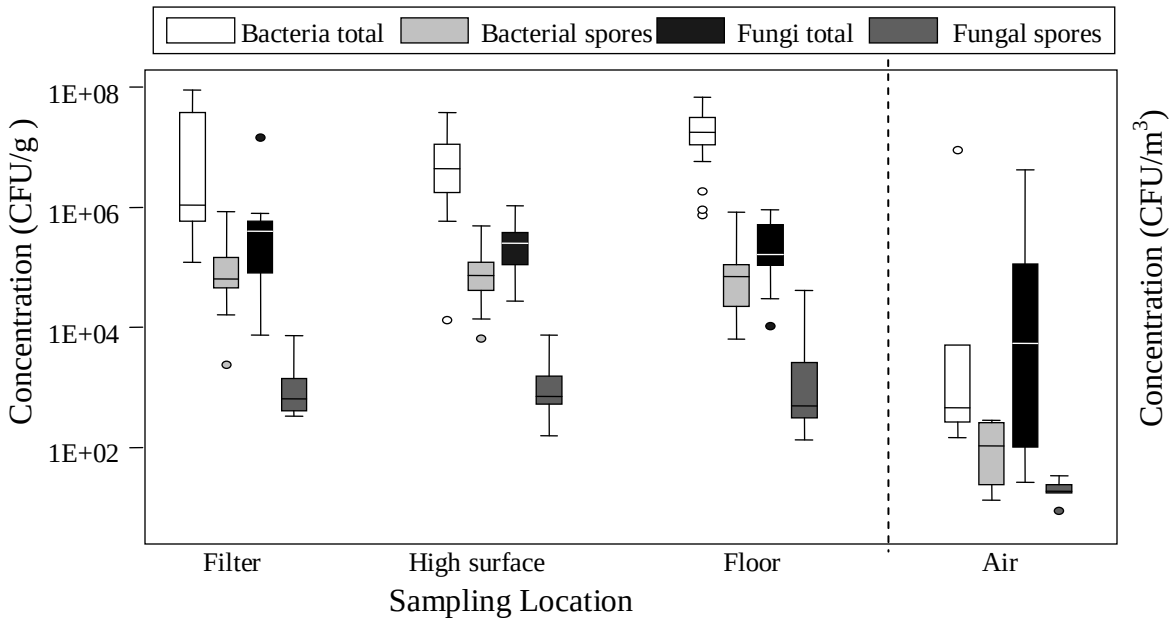


Figure 1. Culturable microbial concentrations by sampling location. Air samples have dimensions of CFU/m^3 and all others have dimensions of CFU/g, with n= number of residences. The lowest end of the box represents the 25^{th} percentile, the top represents the 75^{th} percentile, and the horizontal bar inside the box indicates the median of the distributions. Single points outside the boxes are outliers.

The culturable concentrations for both fungi and bacteria depicted in Figure 1 are generally consistent with the published literature. Indoor concentrations for bacteria and fungi vary considerably with reported values ranging from 10^2 to 10^4 CFU/m^3 for indoor air and from 10^3 to 10^7 CFU/g^1 for settled dust (Andersson *et al.*, 1999; Bouillard *et al.*, 2005; Dales *et al.*, 1997; Koch *et al.*, 2000; Ren *et al.*, 1999; Ross *et al.*, 2000). For all of the sampling locations, the observed viable bacterial concentrations are higher than those for the fungal concentrations and the estimated spore concentrations are approximately two orders of magnitude lower than total concentrations. Total bacteria concentrations range from 10^4 to 10^7 CFU/g^1, with a greater median concentration found on the floor,

followed by high surfaces, and HVAC filter samples with median concentrations of 1.9×10^7, 4.4×10^6 and 1.1×10^6 CFU/g, respectively. This suggests that larger particles or clusters of bacterial cells that are more likely to settle may have greater bacterial concentrations than small particles that remain suspended in air and are captured on the filter. Another possible explanation could be that the survival/growth conditions and nutrient availability on surfaces may be more favorable than on filters. Fungal concentrations in the dust samples ranged from 10^3 to 10^7 CFU g^{-1} with reasonably consistent distributions across the dust sampling locations. It is important to note that airborne microorganisms may be attached to particles, and the size of the particles to which they are attached may have the greatest influence on their fate in an indoor environment (Hairston *et al.*, 1997).

A greater variation in fungal spore concentrations was observed in the floor dust samples, possibly due to the different types of flooring (i.e., carpet and hardwood floor) present in the different residences. However, there was a small variation in the bacterial concentrations suggesting that other factors beside floor surface characteristics may be important. In the air samples, there the median fungal concentration was greater than the median bacterial concentration and the concentration of culturable fungi varied considerably. Several other studies have found that indoor air fungal concentrations have elevated temporal and spatial variability (Hyvärinen *et al.*, 2001; Koch *et al.*, 2000), and thus the short sampling time utilized in the current study may have affected the results. Stanley *et al.* (2008) calculated low culturable concentrations for selected bacterial species in indoor air, often below 4 CFU/m^3, based on HVAC filter dust concentrations. The results in the current study diverge from those perhaps due to differences in quantification techniques and the fact that HVAC systems in the current study supplied

100% recirculated indoor air and operated intermittently when the thermostat called for conditioning.

The mean culturable concentrations of bacteria and fungi in the floor dust, high surface dust and HVAC dust at each of the eight residences investigated are summarized in Figure 2. The concentration of bacteria was fairly consistent within one order of magnitude across most sites except for Sites 1, 2 and 3. At Site 3, HVAC filter and high surface dust concentrations were quite similar but the floor dust samples had much greater concentrations and may have been influenced by tracked-in particles. The difference between the HVAC filter and the high surface dust samples at Sites 1 and 2 may be due to the reduced efficiency of the filters collected from these sites, specifically one low- and one mid-efficiency filter for Site 1 and two low-efficiency filters for Site 2. The reduced efficiency of these filters may make them less ideal sampling devices and increases the probability of observing differing microbial concentrations on the filters than on the floor or high surface. The difference in microbial concentrations on the filters and those found in surface and floor dust at these three sites may also be attributable to the cycling of the HVAC system (Appendix A), suggesting that HVAC filters in residential buildings where the HVAC system is operated sporadically may be less representative of indoor contaminant concentrations. Nevertheless, despite some site-specific differences, the Wilcoxon sign-rank test reveals that the culturable microbial concentrations encountered at different dust sampling locations across all the sites were not statistically different.

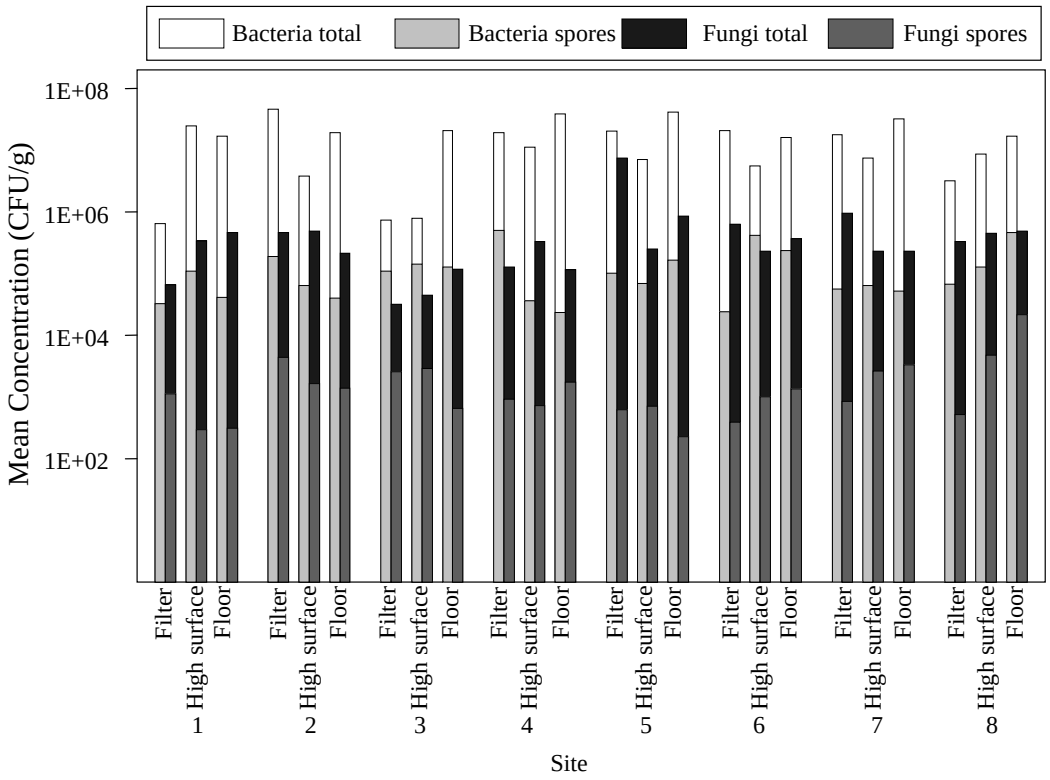


Figure 2. Mean culturable microbial concentrations in dust samples by location within the buildings.

From Figures 1 and 2 it is clear that both bacteria and fungi are able to populate and survive in the dust present indoors. Importantly for this work, in a humid and warm environment like central Texas during the cooling season, microorganisms appear to survive and colonize the dust on HVAC filters with concentrations similar to those found in the dust that settles inside the residences, suggesting that these filters may be a promising location for collecting samples for indoor assessments. While the culturable concentrations were comparable, the compositions of the microbial communities may differ with sampling location because of specific environmental conditions that may

favor some species over others. This aspect, as well as the influence of occupants on the composition of indoor microbial communities, is addressed in Phase II.

The median microbial concentrations observed on filters with different MERV ratings are summarized in Table 2. Median microbial concentrations on HVAC filters were relatively consistent across filters with different removal efficiencies. The median concentrations were typically within one order of magnitude of each other and application of the Wilcoxon Rank-Sum Test to the data did not find any significant differences between filters with different MERV ratings.

Table 2. Median microbial concentrations (with uncertainty) in HVAC filter dust for filters with different efficiencies.

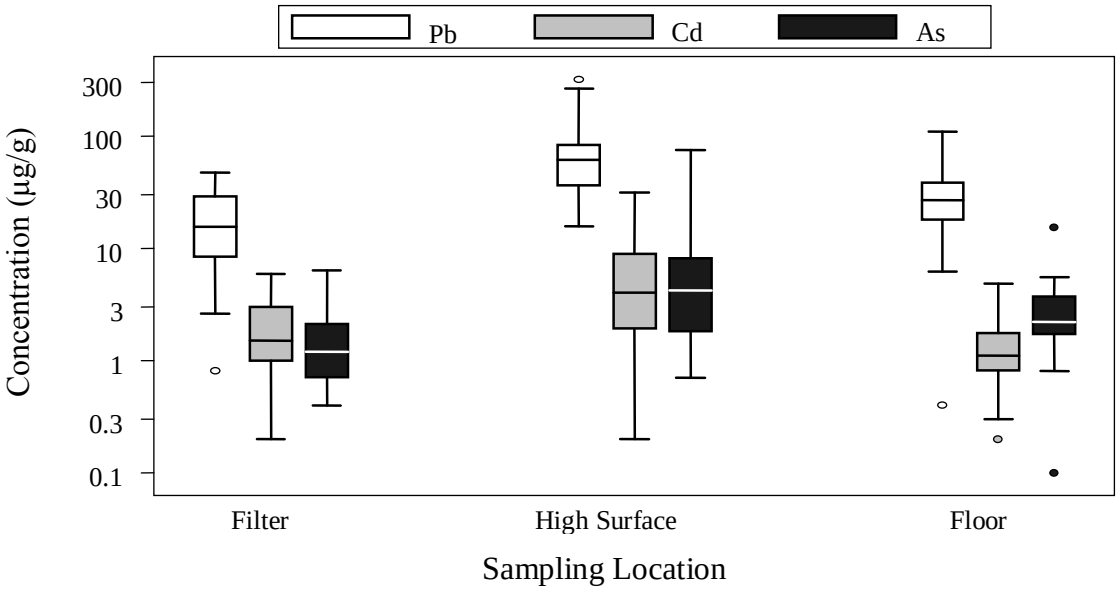
Filter MERV	Bacteria	Bacterial spores	Fungi	Fungal spores
	CFU/g			
Low	$6\times10^{6} \pm 6\times10^{5}$	$5\times10^{4} \pm 9\times10^{3}$	$4\times10^{5} \pm 7\times10^{4}$	$1\times10^{3} \pm 1\times10^{3}$
Mid	$9\times10^{5} \pm 2\times10^{5}$	$7\times10^{4} \pm 7\times10^{3}$	$6\times10^{5} \pm 1\times10^{5}$	$8\times10^{2} \pm 1\times10^{3}$
High	$3\times10^{5} \pm 6\times10^{4}$	$7\times10^{4} \pm 3\times10^{3}$	$1\times10^{5} \pm 9\times10^{4}$	$6\times10^{2} \pm 7\times10^{2}$

The concentrations of Pb, Cd and As at the different residential sampling locations is presented in Figure 3. Of the three metals, Pb was present in the highest concentrations at all three locations with a total median concentration of 30.9 μg/g and values as high as 315 μg/g. This is consistent with previous studies in which Pb has been reported to be abundant in indoor dust (Oliver *et al.*, 1999). Cd and As have comparable profiles with much lower median concentrations of 1.6 and 1.3 μg/g, respectively. However, As has greater variability than Cd with values up to 75.2 μg/g. The mean HVAC filter dust concentrations for Pb, Cd and As were 13.0, 1.9 and 1.4 μg/g, respectively.

The metal concentrations reported in the literature for indoor dust are similar to those reported here for HVAC filter dust and are typically in the μg/g range (Al-Rajhi *et*

al., 1996; Lisiewicz *et al.*, 2000; Turner *et al.*, 2006). Sites 5, 6 and 7 had higher HVAC filter Pb concentrations than did the rest of the samples. None of the three sites has attached garages or is located adjacent to a major highway, suggesting that leaded gasoline is not the major source of indoor lead. Sites 5 and 6 were the oldest sites investigated and we hypothesize that the elevated Pb concentration was derived from lead-based paint, still in use when the residences were built. Several researchers (Chattopadhyay *et al.*, 2003; Kim *et al.*, 1998; Tong, 1998) provide evidence for this hypothesis. There was uncertainty about the age of Site 7, the other site with an elevated Pb concentration although it was located in a neighborhood constructed in the 1970s and was likely to have contained leaded paint. Site 3, the newest residence investigated, had the lowest Pb concentration, supporting the argument that leaded paint is an important contributor to indoor lead concentrations. A correlation between the age of a property and Pb concentrations in settled dust has also been observed by other researchers (Adgate *et al.*, 1998; Kim *et al.*, 1998; Tong, 1998). However this association is not entirely consistent throughout our study; for instance, Site 4, which is also a new residence, had a higher Pb concentration than did several older sites in the study so other factors including localized sources and zoning of the building may be important.

The metal concentrations found in the high surface samples are greater than those present in the other two locations for all three metals. Specifically, filter dust concentrations were statistically lower ($p<0.05$) than those from high surface dust for all metals and statistically lower than those from floor dust for all metals except Cd. Considering that larger particles may be more likely to settle on surfaces than to stay suspended in air, this would suggest that large particles may have a greater concentration of heavy metals and may be associated with metal sources, as reported by Al-Rajhi *et al.*



(1996). Lower concentrations on floor dust were observed. However, floor dust may not be of indoor origin and could have been carried on the building floor from outside.

Figure 3. Heavy metal concentrations by sampling location.

The median concentrations of Pb, Cd and As in HVAC filter dust collected on filters with different removal efficiencies are summarized in Table 3. The median metal concentrations for the high-efficiency and low-efficiency filters were always the lowest and the greatest, respectively. For Pb and As, the concentrations in the high-efficiency filters were significantly lower than those in the low-efficiency filters. In our study, Cd concentrations were reasonably uniform across filters with different efficiencies and the Cd concentrations detected were comparable to values reported in the literature for settled dust (Jaradat *et al.*, 2004; Momani *et al.*, 2002; Turner *et al.*, 2006). Based on the MERV classification (ASHRAE, 2007), low efficiency filters collect a greater fraction of large particles than high efficiency filters supporting the suggestion that large particle size fractions may be associated with greater metal concentrations. This observation is in

accordance with the findings of Al-Rajhi *et al.* (1996). However, Lisiewicz *et al.*, (2000) detected higher metal concentrations in fine particles collected from indoor floors than in larger particles.

From this study, general conclusions are difficult to draw because of the limited number of sites investigated and because the filters with different efficiencies were not uniformly distributed throughout the sites. Therefore, some biases due to potential site-specific sources were possible. Furthermore, different metal sources, both indoor and outdoor, may have a significant influence on the metal concentration distribution for particles of different sizes. At the same time, since high efficiency filters collect a greater mass of particles than low efficiency filters, they remove a greater amount of metals from the indoor environment

Table 3. Median metal concentrations in the HVAC dust for filters with different efficiencies.

Filter MERV	Pb	Cd	As
		µg/g	
Low	18.5 ± 0.79	2.00 ± 0.062	4.61 ± 0.24
Mid	12.9 ± 0.54	1.64 ± 0.035	1.89 ± 0.32
High	7.49 ± 0.44	1.54 ± 0.027	0.912 ± 0.22

During this field investigation we observed the presence of several confounding factors including filter efficiency, HVAC system cycling and the importance of particle size. The role of these factors in the application of HVAC filters as a sampling tool will be addressed in Phase III.

4.2 MICROBIAL COMMUNITY INVESTIGATION

Following the investigation of bacterial and fungal culturable concentrations in residential sites, the study was then expanded to a DNA-based analysis of the microbial communities present in a subset of four residential sites and in a mostly unoccupied full-

scale test house. The objective of this phase of the dissertation was to evaluate the use of HVAC filters as samplers for indoor microbial community analysis. The characterization of the communities is particularly important since similar microbial concentrations could still mean different levels of contamination due to the presence of specific species that thrive in certain environments. The comparison between the communities will provide information regarding the validity of using HVAC filters as representative samplers of indoor microbial communities. In addition, the identification of microbial species common to HVAC filters and the association between occupants and microbial species could also be investigated.

After eliminating all potentially chimeric or poor quality sequences, we obtained a total of 915 bacterial clones and 919 fungal clones, corresponding to 248 and 295 OTUs, respectively. The bacterial clones had an overall Chao1 value of 426 and a Shannon-Wiener index of 4.58, while fungal clones had values of 508 and 4.62, respectively. These values indicate a microbial representation similar to that observed in other indoor studies (Pitkäranta *et al.*, 2008; Rintala *et al.*, 2008; Täubel *et al.*, 2009) confirming the diverse bacterial and fungal communities present in indoor environments. In these other indoor studies, Rintala *et al.* (2008) focused on the influence of seasons on the compositions of floor dust bacterial communities in two commercial buildings, while Täubel *et al.* (2009) investigated the source of bacterial house dust in four residential buildings. Pitkäranta *et al.* (2008) studied the fungal communities in two office buildings using different techniques. In these three studies the overall Shannon-Wiener ranged from 1.92 to 4.22, while the Chao1 ranged from 339 to 464.

The bacterial composition at the phylum level for all the samples analyzed is summarized in Figure 4. This composition is based on the assumption that all microorganisms present in the samples have the same likelihood of being detected and

identified in the clone library. However, biases are possible and the DNA of certain species may be extracted, amplified or cloned more easily than others causing potential biases in the analysis. Nevertheless, the prevalence of a specific microorganism in the clone library generated from a given sample can be used to estimate its relative occurrence in a sample (Lane *et al.*, 1985). The most common phyla encountered in the clone libraries were gram-negative Proteobacteria, and gram-positive Actinobacteria and Firmicutes, a finding which is in agreement with recent DNA-based studies by Rintala *et al.* (2008) and Täubel *et al.* (2009). These three phyla represent 96% of the clones encountered on the residential filters and 90% of the clones found in the high surface residential samples in the current study. For all the residential sites investigated, Proteobacteria were present in greater proportion in the filter dust samples than in the high surface samples, with mean values of 65% and 39%, respectively. Tringe *et al.* (2008) utilized a DNA-based technique similar to the current study and also observed an elevated proportion of Proteobacteria on HVAC filters in two commercial buildings. These results contrast to those reported by Stanley *et al.* (2008) who observed that the gram positive *Bacillus* (of the Firmicutes phylum) was the most commonly identified group in a culture-based study of HVAC filter bacterial communities. Thus, the prevalence of gram-positive bacteria in the Stanley *et al.* (2008) study may be due to a bias of culturing techniques that favor gram-positive bacteria. The results from culture-independent studies described herein and by others suggest that Proteobacteria represent a significant fraction of the indoor air bacterial community and that this phylum may better tolerate the environmental conditions encountered in air (Brodie *et al.*, 2006; Fierer *et al.*, 2008) and on HVAC filters. One explanation could be that they possess a greater fraction of key genes involved with resistance to desiccation and oxidative damage, as reported by Tringe *et al.*, (2008). While Proteobacteria (mainly *Ralstonia*, *Pantoea* and

Enterobacter spp.) dominated the filter dust samples, an opposite trend was observed for Actinobacteria, with the mean percentage in the high surface samples more than four times higher than that found on the filters, i.e. 26% versus 6%.

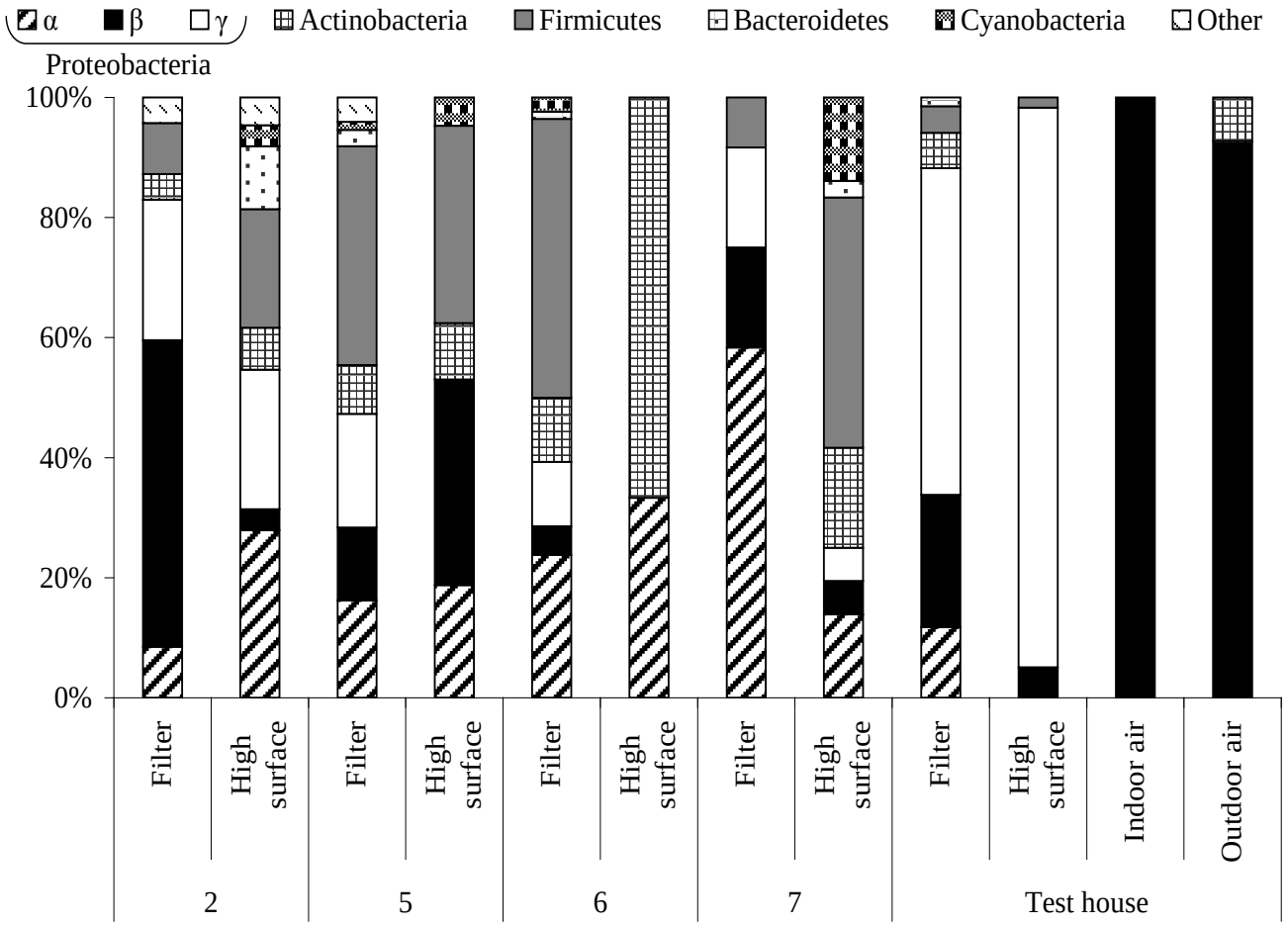


Figure 4. Bacterial composition at the phylum level for the sequence libraries obtained from all the samples analyzed in the four residences (Site 2, 5, 6, and 7) and in the test house.

Comparison of the clone libraries generated from the dust samples in occupied residences to those in the unoccupied test house indicates that a much greater proportion of gram-positive bacteria, mainly Firmicutes and Actinobacteria, were present in the residences versus in the test house, with mean values of 41% and 6%, respectively. This

increased proportion of gram-positive bacteria in occupied buildings supports the speculation that many gram-positive bacteria found indoors may be attributable to human sources (Horak *et al.*, 1996; Pakarinen *et al.*, 2008; Rintala *et al.*, 2008; Täubel *et al.*, 2009). Rintala *et al.* (2008) examined the bacterial communities in surface dust in two buildings across seasons. They observed higher variation in microbial composition between buildings than between seasons, suggesting the development of site-specific bacterial communities, and that building users may be responsible for the presence of the dominant bacterial groups. A similar suggestion was made by Täubel *et al.* (2009) after examining the bacterial communities in mattress dust, floor dust and skin surface samples of occupants in four residences.

In the current investigation, we observed a greater proportion of gram-negative bacteria, primarily Proteobacteria, in the dust samples collected in the test house (mean value of 93%) versus those collected in the residences (52%), corroborating the supposition that gram-negative bacteria, and specifically Proteobacteria, may be of outdoor (i.e., environmental) origin. In the test house, we observed a dominance of Proteobacteria for all the samples analyzed. Fierer *et al.* (2008) reported elevated temporal variability in outdoor samples with a dominance, across the five sampling days, of Proteobacteria and Bacteroidetes. This latter phylum was rarely observed in the current study and may be more typical of colder climates (Miteva *et al.*, 2004; Yi *et al.*, 2005). One possible reason is that Bacteriodetes may have the ability to survive in inhospitable and highly oligotrophic environments (Fierer *et al.*, 2008). An elevated presence of Proteobacteria in outdoor air communities was also reported by Brodie *et al.* (2006) for the same geographic area of this study, confirming that this may be the most abundant phylum in ambient air samples.

The fungal composition at the subclass level for all the clone libraries acquired is shown in Figure 5. The majority of the sequences belonged to the phylum Ascomycota, with a much smaller fraction assigned to the Basidiomycota phylum. The majority of the fungal clones encountered in the samples analyzed belong to the *Dothideomycetes* (*Pleosporomycetidae*, *Dothideomycetidae* subclasses), *Sordariomycetes* (*Hypocreomycetidae*, *Sordariomycetes incertae sedis*) or *Agaricomycetes* (*Agaricomycetes incertae sedis*) class. Specifically, the Dothideomycetes class seems to be dominant, with *Cladosporium* and *Alternaria* spp. being the most abundant representatives. Pitkäranta *et al.* (2008) also observed an abundance of the Dothideomycetes class in indoor dust from two nursing homes in Finland even though the most common phylum was Basidiomycota. However, they also observed an increase in *Dothideomycetes*, and therefore in Ascomycota, during the summer months which represent a more similar climate to that encountered in central Texas in summer and fall. In the *Sordariomycetes* class, members of the genera *Fusarium* spp. were the most commonly detected, which is consistent with results of other studies (O'Brien *et al.*, 2005; Pitkäranta *et al.*, 2008) that also used a molecular-based approach. Some culture-based studies have reported elevated concentrations of the genera *Penicillium* and *Aspergillus* spp. in indoor and outdoor communities (Koch *et al.*, 2000; Ren *et al.*, 1999). However, in the current study, we observed a limited proportion of the class corresponding to these genera, Eurotiomycetes. This discrepancy could be due to a specific bias of the culturing methods that favor these species.

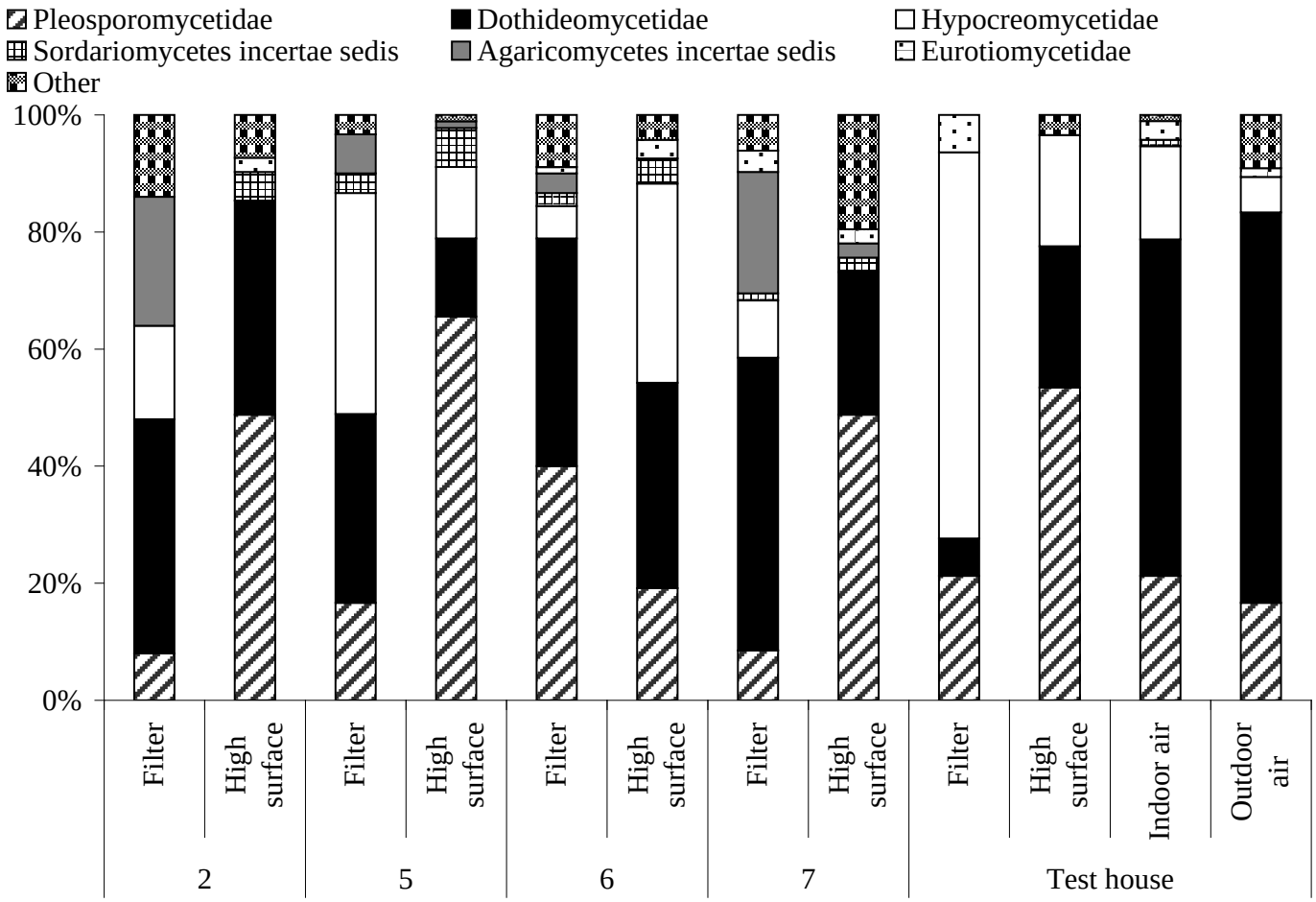


Figure 5. Fungal composition at the subclass level for the sequence libraries obtained from all the samples analyzed in the four residences (Site 2, 5, 6, and 7) and in the test house.

When comparing the fungal composition of a given site, it was observed that for all the sites except Site 6 the proportion of Dothideomycetes was greater in high surface dust (a mean of 76%) than in filter dust samples (a mean of 59%). An opposite behavior is observed for Agaricomycetes that are present in filter dust samples in much greater proportion than in high surface samples for all the residential sites, with mean values of 16% and 1%, respectively. Sordariomycetes are present in a greater proportion in filters than in the high surface dust samples. This is especially true for the test house filter,

where this class seems to proliferate constituting 66% of the fungal clones obtained. The proportion of Sordariomycetes increased from a mean value of 19% to 42% among all the dust samples in the residences and those in the test house. This class has been observed to dominate in outdoor air samples (Fierer *et al.*, 2008) confirming the potential environmental origin of this class in the test house. However, the fraction of this class was not particularly high in indoor air, but seems to proliferate in the test house filter. Therefore other factors may be important. Both indoor and outdoor air samples were dominated by ascomycetes, as also reported by Fierer *et al.* (2008) for outdoor air, supporting the hypothesis that indoor fungal communities strongly depend on outdoor fungal microbiota (Pitkäranta *et al.*, 2008).

Some of the clones from the occupied residences have high similarity to species that are reported to be potential opportunistic pathogens. These species include for Bacteria *Pantoea agglomerans*, *Ralstonia pickettii*, *Enterobacter hormaechei*, *Staphylococcus aureus* and *epidermidis*, as well as *Bacillus cereus*, *pumilus*, and *subtilus*. Fungal potential pathogens include *Alternaria alternate* and *tenuissima*, *Fusarium proliferatum* and *oxysporum*, *Nigrospora shaerica*, and *Cladosporium cladosporioide.*, The presence of these opportunistic pathogens on HVAC filters confirm the potential application of filters as samplers for detecting harmful microorganisms. However, additional analyses will be required to determine if these microbes were actually in a viable state.

To evaluate the potential use of HVAC filters as a sampling mechanism for indoor microbial communities, the similarity between microbial communities in different indoor sampling locations was evaluated using the UniFrac significance metric. The comparison between communities using the phyla percentage composition illustrated previously is an effective and visual way to classify the microorganisms encountered.

However, it has limitations including the fact that similar percentage compositions may not necessarily mean that similar species are present. Species in the same phylum could be either extremely similar or quite distant from a phylogenetic standpoint. The same could be true for the opposite example, where different compositions in terms of phyla/classes could illustrate communities extremely different or rather similar, depending on the distance in evolution of the species present in the communities. Table 4 presents the UniFrac values for the comparisons between the HVAC filter and high surface dust samples in the residential sites. From the p-values, it appears that, although some differences in composition are present (Figs. 4 and 5), both bacterial and fungal communities in the filter and high surface dust samples within each residence investigated are not statistically different. Thus, the UniFrac results suggest that in a given residence, the microbial community present in high surface dust is similar to that present in HVAC filter dust and high-efficiency filters may be suitable samplers for assessing the composition of indoor microbial communities. This similarity seems to contradict some of the compositional findings described above, possibly because of the nature of the Unifrac analysis, which relies on a phylogenetic distance comparison rather than a species by species comparison.

While the UniFrac comparisons indicate that the microbial communities in HVAC filter dust and high surface dust are similar in a given residence, Site 7 had the lowest p-values of all the residences for both the bacterial and fungal community comparisons, with a bacteria p-value of 0.10, the threshold of significance. One possible explanation is the location of the HVAC filter in this residence. The filter was located at the return grille in the hallway, away from some of the rooms where the high surface dust sample was collected (living room and two of the four bedrooms) potentially resulting in differences in the particles collected on the filter versus those being deposited on indoor

surfaces. The fungal and bacterial concentrations determined using culturing techniques (Fig. 2) were similar for the filter and high surface dusts at Site 7, while greater variations in the microbial concentrations between filter and high surface dusts were observed at other sites (i.e., bacteria for Site 2, fungi for Site 5) that had similar microbial communities (Table 4). This lack of correlation between culture-based and culture-independent results confirms the fact that similarity in culturable concentrations is not necessarily associated with similarities in the microbial communities. Thus, the communities need to be characterized using a culture-independent technique. When we monitored the HVAC system usage for 2-3 day-long monitoring periods during Phase I (Appendix A), Site 7 had the highest cooling duty cycle (34% compared to a median of 18% for the other sites), suggesting that other factors other than the HVAC system usage could be important. More intuitively, for the other two sites where a high cooling duty cycle was found, Sites 5 (32%) and 6 (29%), the p-values are well above the threshold. However, caution is suggested in interpreting these results because of lack of direct monitoring of HVAC operation during the measurements described here.

Table 4. UniFrac significance values for the filter to high surface comparison in the four residential sites.

	UniFrac Significance (P-value)			
	Site number			
	2	5	6	7
Bacteria	0.41	0.35	0.48	0.10
Fungi	0.30	0.65	0.55	0.16

The UniFrac significance values for the samples collected in the unoccupied full-scale test house are shown in Figure 5. The p-values for the microbial community comparisons between the filter and high surface dust samples (second column) are lower than those determined for the residences (Table 4), suggesting that in the unoccupied test

house the microbial communities in these two sampling locations are more distinct from each other. In contrast, human-associated microorganisms seem to dominate the communities in occupied residences (Rintala *et al.*, 2008; Täubel *et al.*, 2009) suggesting that that occupied residences have a more homogeneous microbial distribution at the different sampling locations. This could be due to the fact that in residences, occupants generate particles through their activities and introduce microorganisms that could deposit onto surfaces or be captured by the filter leading to a more homogeneous distribution of microorganisms in the indoor environment. In contrast, the fungal communities in the HVAC filter and high surface dust samples in the unoccupied test house are statistically different, while bacteria are not, suggesting the fungi may be more prone than bacteria to develop communities adapted to the specific environment. In addition, another possible explanation is that fungi may be more likely to grow and multiply on filters leading to a shift in the filter microbial community relative to the indoor air. The difference in the fungal communities observed in filter and high surface dust in the test house could be attributable to the increased proportion of Sordariomycetes in filter dust, supporting the assumption that this class may be of outdoor origin (Fierer *et al.*, 2008).

Table 5 UniFrac significance values for the samples collected in the test house

	UniFrac Significance (P-value)			
	Filter vs. high surface	Filter vs. indoor air	High surface vs. indoor air	Indoor vs. outdoor air
Bacteria	0.13	0.14	**0.01**	0.12
Fungi	**0.01**	0.24	**0.03**	0.19

The results from the month-long investigation in the unoccupied test house indicate that the filter and the composite indoor air sample (third column) were not statistically different, supporting the findings of Tringe *et al.* (2008) that suggested that

HVAC filter dust can be used as an integrated measure of airborne microbial communities, even though some specific differences in the community may occur. HVAC filters are in place for extended periods of time. Therefore, during their usage a large volume of air is filtered through them (Stanley *et al.*, 2008) and only the microorganisms that were, at some point in time, airborne have the opportunity to be captured on the filter. In this study, the airborne microbial communities derived from the analysis of a composite sample of 20 daily 1-hour samples represent a more integrated measurement. Shorter-term air samples are reported to have a great temporal variability (Brodie *et al.*, 2006; Fierer *et al.*, 2008) and by compositing daily collections we tried to overcome this limitation so as to be able to compare the indoor air samples to the dust that collected on the surfaces and on the filter over the same period.

The values reported in the fourth column of Table 5 indicate that both bacterial and fungal communities in high surface dust and indoor air were statistically different in the unoccupied test house. Therefore, high surface dust samples are not representative of airborne microbial communities, possibly because surface dust samples may be influenced by the microorganisms attached to the particles that are more likely to deposit on surfaces (i.e., larger particles) rather than stay in air. Single bacterial and fungal cells range from 0.5 μm to 50 μm, with fungal spores generally larger than bacterial spores (Li and Li, 1996; Terzieva *et al.*, 1996). Also both bacteria and fungi are often associated with particles which alter their effective size. The size of these biological particles influences their fate and the probability of being detected in the different sampling locations, since larger particles are likely to settle while smaller particles may stay longer in air and have more opportunities to be captured on HVAC filters. In the residences, the presence of occupants and the microorganisms associated with them tend to homogenize the communities. In the test house, the different fates of particles of various sizes are

more evident due to the limited occupancy. In addition, the microbial communities encountered may be influenced by the different sampling technique employed for settled dust and air. The microbial communities detected on high surfaces depends on the microorganisms (present as single cells, clusters or attached to particles) that are recovered using the vacuum mechanism, and it could be possible that some small particle/cells may remain on the surface and are not captured. In contrast, the impinger method used for air sampling is reported to have elevated collection efficiency for a wide range of particle size (above 90% for particles larger than 0.5μm).

The significantly different bacterial community between these high surface and indoor air samples in the test house seems to be largely attributable to the different composition of Proteobacteria. Air samples were dominated by β-Proteobacteria constituting 100% and 93% of the clone libraries for the indoor and outdoor air samples, respectively. In contrast, the dust samples in the unoccuppied test house are dominated by γ-Proteobacteria which constituted 54% of the bacterial clones encountered in the filter dust and 93% of those found in the high surface dust sample (Fig. 4). For fungi, the difference between high surface and air may reside in the composition of the Dothideomycetes class since 53 % of the clones in the high surface sample belonged to the subclass Pleosporomycetidae, while 24% to the subclass Dothideomycetidae. For indoor air the proportion for these two subclasses is inverted, with the former accounting for 21% and the latter 57% of the sequences (Fig. 5). Finally, we observed similar microbial communities in indoor and outdoor air (last column of Table 5). Most importantly for this investigation, the findings reported in Table 5 confirm that high-efficiency HVAC filters located in HVAC systems operating a great fraction of time can be used as a surrogate for long-term air samples. This suggests that HVAC filters can be used as an alternative to extensive periodic air sample collections and yield statistically

similar information. Given the results from the four occupied sites, we would anticipate that these results would also hold for occupied environments.

4.3 FATE ANALYSIS OF INDOOR PARTICLES

In Phase I and II, we observed the presence of several confounding factors that may play a critical role in the application of HVAC filters as a sampling mechanism. These parameters, including HVAC filter efficiency, HVAC cycling, tightness of the building as well as the influence of particle size, deserved further investigation. As a consequence, in Phase III we focused our attention on the investigation of the fate of indoor airborne particles and the importance of the various removal mechanisms with the objective of evaluating the likelihood of filters to be effective samplers for particle-bound contaminants.

4.3.1 Results from validation experiments

The air exchange rates during the experiments ranged from 1.57 to 1.96 h^{-1}, while the HVAC system flow rate (Q_r) was approximately 1530 m^3/h and 1630 m^3/h for the high and low efficiency filters, respectively. A comparison between the fraction of the injected dust mass that was collected on the filters and the particle volume fraction calculated using Equation 4 is shown in Table 6. Since we assumed a constant density across particle size ranges, the mass fraction and the volume fraction represent the same metric and can be compared. The volume percentage estimated using Equation 4 matches the measured mass fraction on the filters within 10% on a relative basis, except for the Test 3 high efficiency filter and the Test 3 low efficiency filter. During these two experiments, the equation overestimated the fraction of the injected particles captured on the filter, possibly because of nonuniform mixing conditions throughout the house. For these two experiments, the standard deviations of the indoor concentrations measured in

the house normalized by the initial concentrations were the highest values of all the experiments. This would lead to a greater variation (positively or negatively) between what is captured on the filter and what is predicted by the model, which assumes a well-mixed condition. The particle mass collected on the floor and high surfaces varied more between tests than did the mass collected on the filters. During the experiments with high efficiency filters, we calculated that between 57% and 76% of the total mass of particles injected in each test deposited on surfaces, while for the low efficiency experiments this percentage was between 67% and 83%.

Table 6. Comparison between measured mass and calculated volume fraction of injected particles on HVAC filters

Test	Measured Mass Fraction (%)	Calculated Volume Fraction (%)[1]
High efficiency filter test 1	20.0	20.9
High efficiency filter test 2	19.2	19.9
High efficiency filter test 3	14.4	18.5
Low efficiency filter test 1	8.47	8.56
Low efficiency filter test 2	9.15	10.8
Low efficiency filter test 3	7.96	10.9

[1]This fraction is calculated using the upstream and downstream filter concentrations and Equations 3 and 4.

A comparison between the filter capture probabilities determined during the experiments (and calculated using Equation 4) and the model prediction for the low and high efficiency filters is presented in Figure 6. The model utilized the actual λ, Q_r and V_T values measured during the experiments; for the black curves the model utilized the deposition loss rate (β) and the filter efficiency (η) values obtained from the literature (Riley *et al.*, 2002; Waring and Siegel, 2008). While the λ, Q_r and V_T parameters are

relatively easy to obtain and measure, measuring β and η requires particle injection tests which are time consuming and more complex to perform. Thus, we were interested in evaluating the applicability of the model if the β and η parameters are not measured directly but rather estimated from literature values. The results shown in Figure 6 indicate that the observed values and the model predictions follow similar trends suggesting that the modeling approach provides a reasonable prediction of the likelihood of particle capture by filters. However, for high efficiency filters the model overestimated particle capture probabilities relative to that measured during the experiments with mean normalized percentage differences between the probabilities calculated during the experiments and the model predictions of 21%, 72%, 63%, 101%, 92%, and 99% for the six size bins considered.

The grey lines in Figure 6 present the model predictions if the measured β and η values are employed. If these two measured parameters are utilized, the model is in much better agreement with the measured filter capture probabilities, particularly for the high efficiency test, suggesting the strong influence of these two parameters in the predictions and the need to accurately estimate them. A comparison between the β and η values obtained from the literature and those measured during the current experiments is presented in Table 7. Significant differences between the β and η values measured during our tests and the literature values (utilized in the model) exist, particularly for the β values for the smaller size bins. The deposition loss rate (β) has been reported to vary significantly depending on several factors including the structure of the house, building conditions, and mixing conditions (Nazaroff *et al.*, 1993; Riley *et al.*, 2002). During our particle decay tests to estimate the β values, eight fans and one ceiling fan were operated to increase the mixing and the obtain well-mixed conditions. This high level of mixing likely caused elevated average velocities in the house that could have increased the

likelihood of particles to collide against a surface and remain attached to it. This phenomenon is likely to be more important for smaller particles that have lower deposition loss rate and tend to stay longer in air, and may be responsible for the elevated difference in β values between the experiments and the literature values for small particle size bins (Table 7). In Figure 6, we notice a greater difference between the predicted and measured filter removal fraction for particles in the 0.3 to 3 μm size range, mainly because for that size range, the β values estimated during our tests were much greater than the values assumed in the model based on literature values (Table 7). Better agreement between the model predictions and the experimental observations was evident for particles greater than 3 μm, where the model may effectively predict what was observed during the experiments. We also observed differences between the filter efficiencies (η) measured during the tests and the literature values used in the model (Waring and Siegel, 2008), particularly for the high efficiency filter. The efficiency values obtained from the literature were for clean new filters and were determined by ASHRAE Standard 52.2 (ASHRAE, 2007). Filters in real systems and conditions may perform differently than that estimated in the Standard 52.2 tests, particularly if they are challenged with particles of a different nature or if bypass occurs.

For low efficiency filters, the model and the experiments are in better agreement, particularly for larger particle sizes ($\geq$ 2 μm), than during the high efficiency tests. In the low efficiency filter scenario, we observed mean normalized percentage differences between the probabilities calculated during the experiments and the model predictions of 81%, 89%, 18%, 31%, 19%, and 23% for the six particle size bins considered. The filter capture probabilities for the low efficiency filter scenario are relatively constant across the size range, around 0.15, revealing that these filters are not likely to oversample a

particle size range and, therefore, show promise as samplers that are not biased towards a specific particle size.

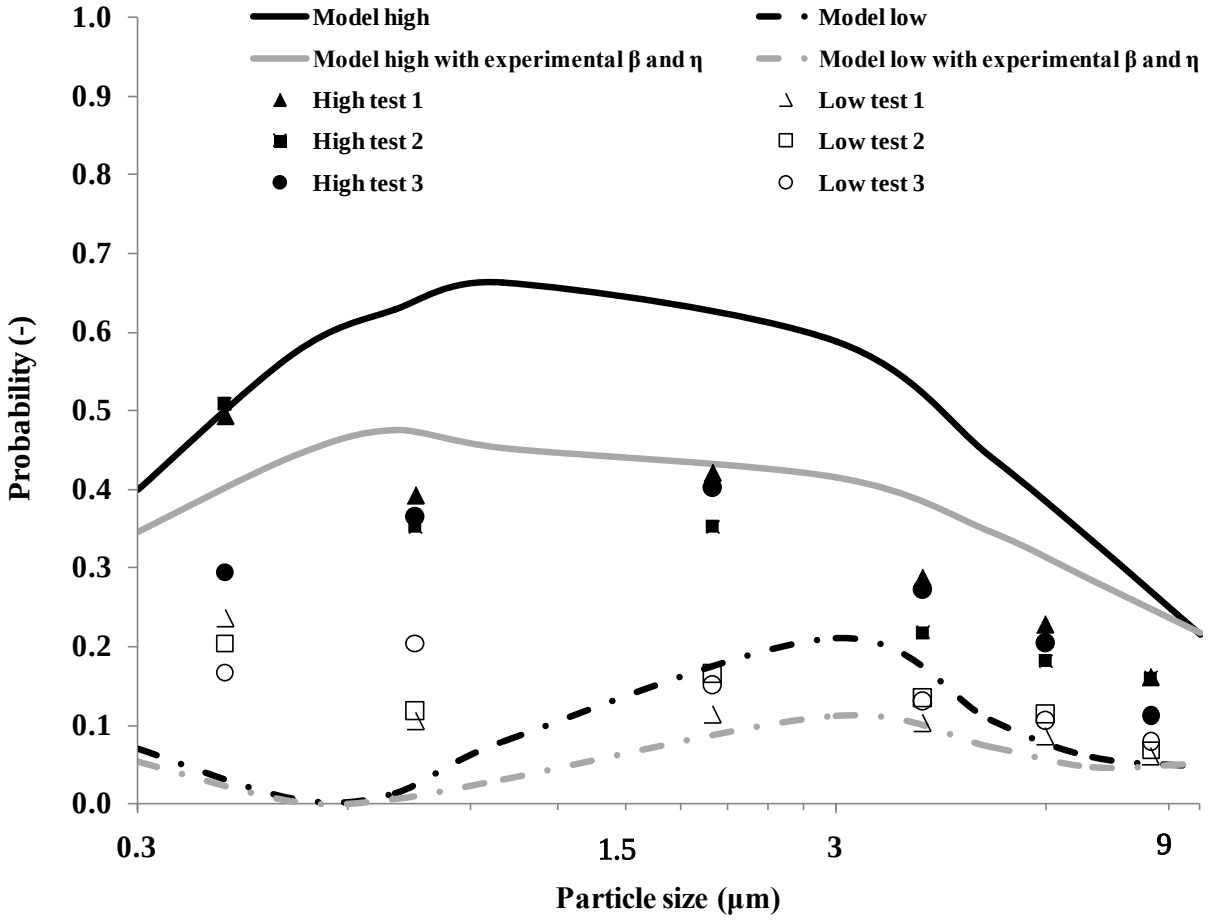


Figure 6. Comparison between model predictions and the observed probabilities during the experiments with the low and high efficiency filters.

Table 7. Comparison between literature and measured values for the β and η coefficients.

Size bin (µm)	β (h^{-1})			η (-)					
				Low efficiency filter			High efficiency filter		
	Literature	Tests	% difference	Literature	Tests	% difference	Literature	Tests	% difference
0.3-0.5	0.03	1.00	97	0.01	0.13	92	0.43	0.33	30

0.5-1	0.05	1.77	97	0.01	0.12	92	0.66	0.58	14
1-3	0.92	4.01	77	0.08	0.11	27	0.86	0.77	12
3-5	3.37	6.88	51	0.13	0.29	55	0.96	0.90	7
5-7	7.10	9.95	28	0.11	0.06	83	0.98	0.92	7
7-10	14.45	14.3	0.8	0.13	0.07	85	0.98	0.96	2

The variations between the model predictions and the experimental results confirm the complexity of the phenomena and suggest that several factors are important and play a role in the fate of particles in an indoor environment. For instance, even with the high level of mixing present during the tests, the assumption of perfectly well-mixed conditions in the house may not have been met and this affects the predicted particle concentrations and capture efficiencies. If the well-mixed assumption is not met, there are lower (or greater) particle concentrations near the return and the particles have actually a smaller (or greater) probability of being captured on the filter than what the model predicts with the assumption of perfectly mixed conditions. Another factor that could have influenced the results is the assumption of constant density across the particle size range. It is possible that larger particles may have greater density because they are more likely to contain crustal material (Seinfeld and Pandis, 1998). The fact that we assumed a lower density for larger particles would lead to an over prediction in the volume (or number) of particles injected, v_i, which would lead to an under prediction in the probability of filter capture, $p_{f,e}$. Moreover, the particles were assumed to be spherical and a characteristic size for each bin was utilized to estimate the volume of the particles measured on that bin. This assumption could have also affected the filter capture probabilities because a different volume of particles could have been injected. In addition, some particles may have coagulated and formed clumped, increasing the

fraction of particles that deposited on surfaces and lowering the fraction that could possibly be captured on the filter.

4.3.2 Model results

Even with the discrepancies described above, the experiments in the full-scale test house indicate that the modeling approach can be utilized to estimate the likelihood that particles are collected on filters or are removed from the air via other mechanisms such as deposition or exfiltration. Subsequently, in order to evaluate a broader application of filters as samplers, the model was applied to more realistic cases and conditions using typical characteristic times for each removal mechanism reported in the literature. The removal probability via different mechanisms for the baseline scenario (MERV 6 filter, λ_r= 1.1 h^{-1} and λ= 0.5 h^{-1}) is presented in Figure 7. The results indicate that, for a mid-efficiency filter, large particles (> 3 µm) are likely to deposit on surfaces and are unlikely to get captured on filters. Therefore, for large particles and contaminants associated with them, filters may not be effective samplers and elevated surfaces could be a more suitable sampling location. The deposition probability increases with size due to the increase in the deposition loss rate (β) for larger particles. Particles in the 0.03-3 µm range are likely to be removed by exfiltration or, if a high efficiency filter is installed, captured on the filter (Figure 8). Particles in this range have a greater residence time in air and, therefore, have a greater opportunity to be captured by the filter. The removal probability increases for ultrafine particles (< 0.1 µm) since the filter efficiency for these particles is greater for all three MERV ratings. Sippola *et al.* (2003) and Zhao *et al.* (2009) reported that large particles (> 1 µm) are likely to deposit on surfaces, while small (submicron) particles tend to be exfiltrated. Two peaks in the filter capture probabilities (Figure 7), at approximately 0.01 and 3 µm. For the baseline scenario conditions, filtration is about 18

times less likely to capture a 0.1 µm particle before it is removed by exfiltration and 12 times less likely to capture a 5 µm particle before it deposits on a surface. For an HVAC system operating only 22% of the time, particles between 0.03 and 1 µm are most likely to be removed by exfiltration and are less likely to be found in settled dust or in the dust that collects on a mid efficiency filter.

From Figure 7, we notice how HVAC filters capture particles over a wide size range and are likely to be effective overall samplers. In contrast, settled dust may be biased toward larger particles that have greater mass, while air samplers may oversample those particles (0.03 - 3 µm range) that have a longer residence time. In addition, although the filter capture probability illustrated in Figure 7 does not seem particularly elevated relative to deposition or exfiltration, it is important to note, despite the higher removal probability for these two mechanisms, it may be more difficult to obtain a representative sample since settled dust and air samples only sample a very small fraction of the total surface or volume in a building at a particular time. In contrast, HVAC filters are typically in place for several weeks to months, and during this period a large volume of air is filtered and a significant portion of the filter dust cake can be analyzed easily.

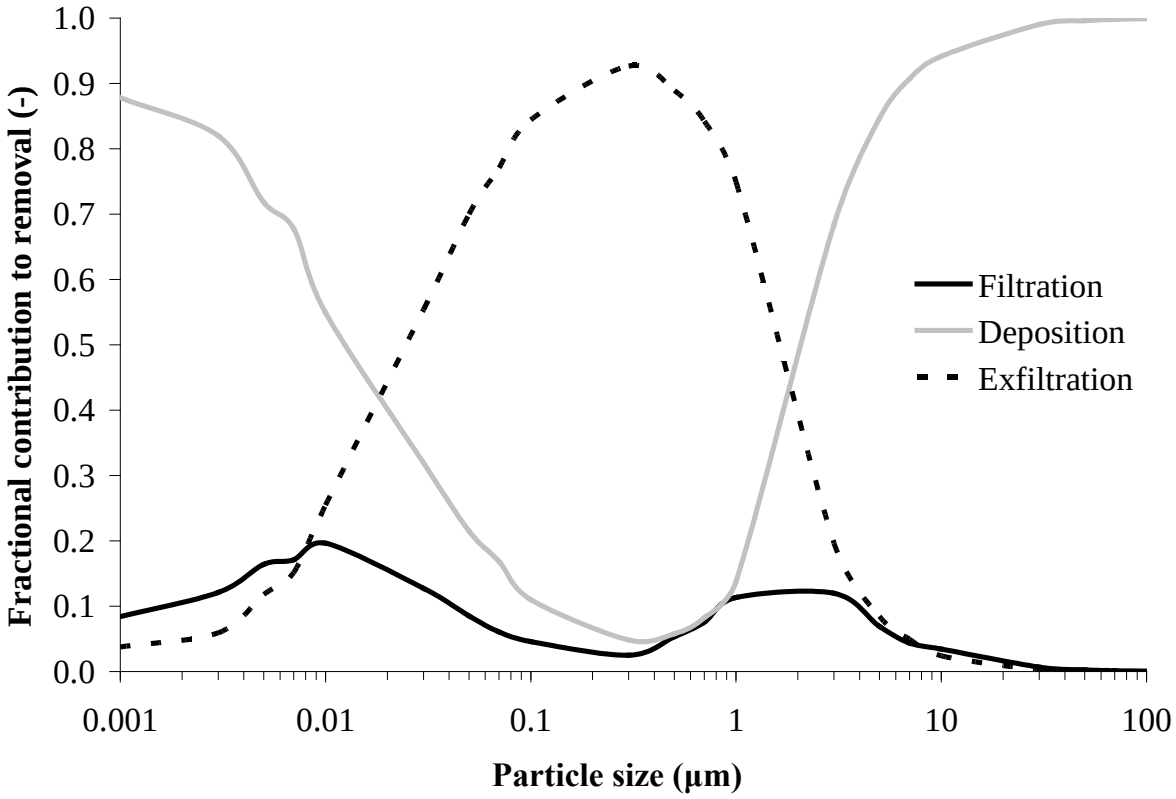


Figure 7. Removal probability curves for filtration, deposition and exfiltration for the baseline scenario (MERV 6, λr=1.1 h^{-1} and λ=0.5 h^{-1}).

Additional results of the analysis are presented in Figures 8, 9, and 10; in each case, one parameter at a time was modified from the baseline scenario. Figure 8 shows that the filter efficiency is a more important variable for sampling particles in the range between 0.03 and 3 µm than for particles below 0.03 µm or above 3 µm. These results are due to the fact that the HVAC filter efficiency is particle-size dependent especially in the range from 0.01 to 3 µm (Hanley *et al.*, 1994). The high efficiency filter probability curve has a minimum around 0.1 µm due to the reduced efficiency of the filter for particles around this dimension. Low and mid efficiency filters have similar probabilities for a wide range of particles, although a greater variation is observed in the range

between 0.3 and 10 µm. If a high efficiency filter is used, there is an elevated probability of capturing particles in the 0.3 – 3 µm and 0.005 – 0.03 µm ranges. In the size range of 0.3 – 3 µm, the model predicts that more than one third of the particles should be captured on a high efficiency filter. As a consequence, high efficiency filters are likely to be reasonable samplers for particles in these size ranges. For larger particles (> 3 µm), deposition onto surfaces is the dominant removal mechanism and filters are less likely to be good samplers.

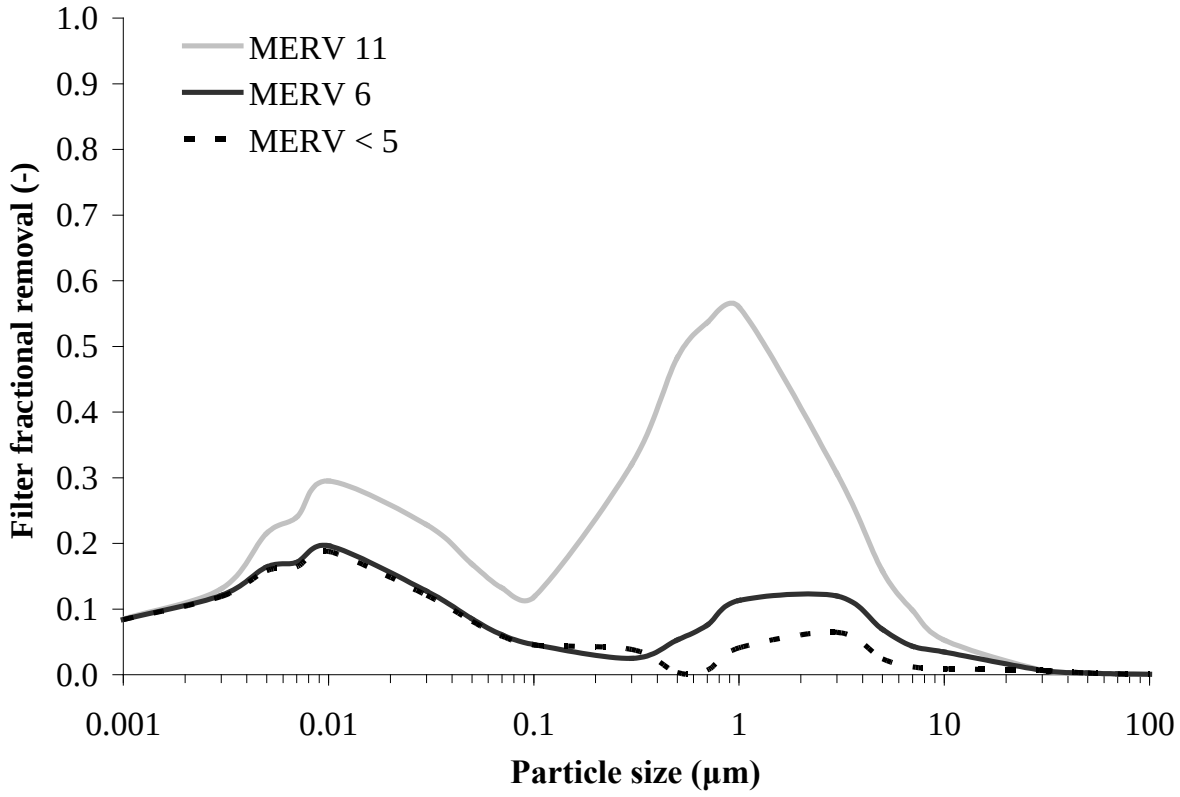


Figure 8. Filter capture probability curves for different filter efficiency scenarios.

Figure 9 illustrates the influence of the HVAC system duty cycle on the filter capture probability curve. The profiles for the two λ_r values investigated follow similar patterns, with the probability for removal via filtration for normal (cycling) use reduced

approximately by the duty cycle fraction (0.22) relative to the continuous operation case. The results suggest that, if a mid efficiency filter is used, the HVAC system would need to operate with an elevated duty cycle in order for the filter to be an effective sampler. However, high efficiency filters with elevated recirculation air exchange rates (> 5.2 h^{-1}) are particularly effective, with more than 30% capture probability up to 7 µm and often above 60% (data not shown). Filters are more effective particle samplers if they have either high removal efficiency or if the HVAC system has an elevated air recirculation rate. If both of these two conditions are met, the filters are more likely than air or settled dust samples to capture particles in a wide size range. In particular, for these conditions, the probabilities of particle capture by the filter are predicted to be as high as 85% for particles around 1 µm.

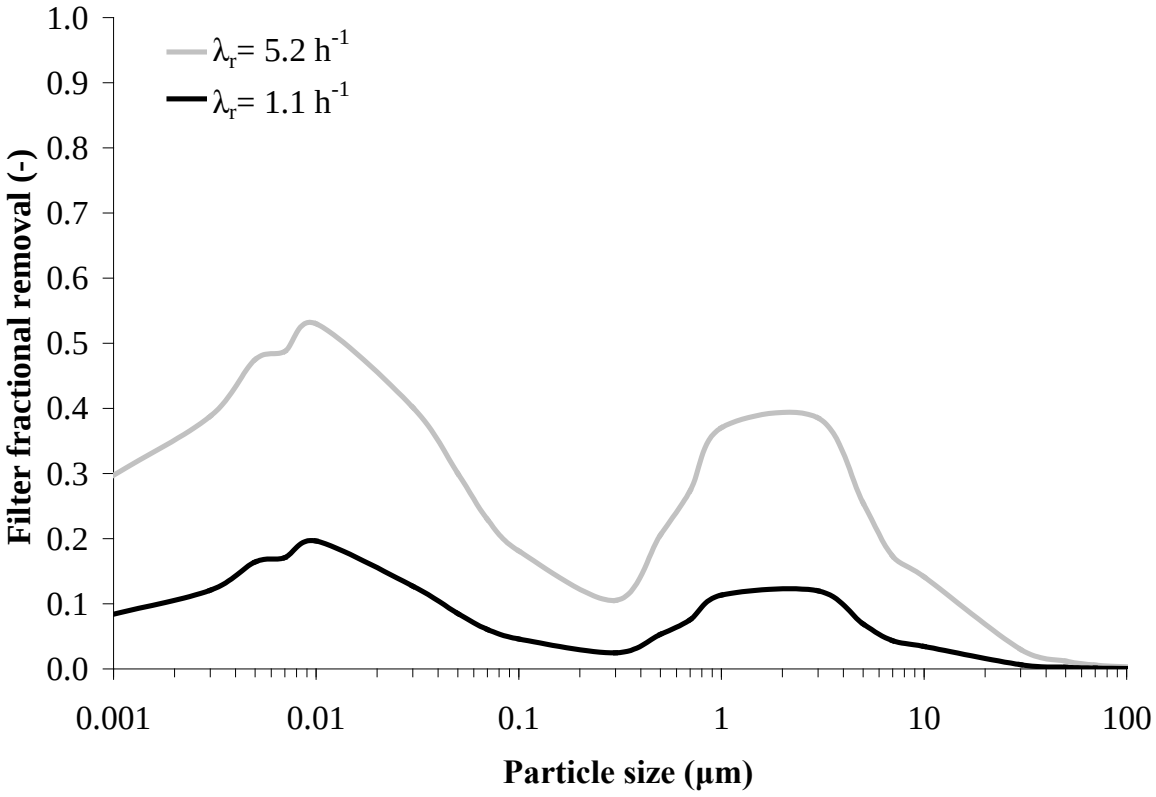


Figure 9. Filter capture probability curves for different air recirculation rate scenarios.

Figure 10 presents the filter probability curves for residences with varying tightness. Abt *el al.* (2000) found that air exchange rate has a significant effect on particle removal. As evident in Figure 10, this parameter does have an influence on the probability of particle capture on the filters; however, this parameter does not seem to be as important as the filter efficiency or the λ_r with similar patterns and capture probability among the three scenarios investigated. For instance, the difference in filter capture probability between a residence with $\lambda = 0.2$ h^{-1} and one with 1.3 h^{-1} is, typically, below 10%. As a consequence, filters could potentially be used as samplers independently of the tightness of the residences investigated. This is an important consideration since the current trend is to move toward tighter and more energy efficient buildings

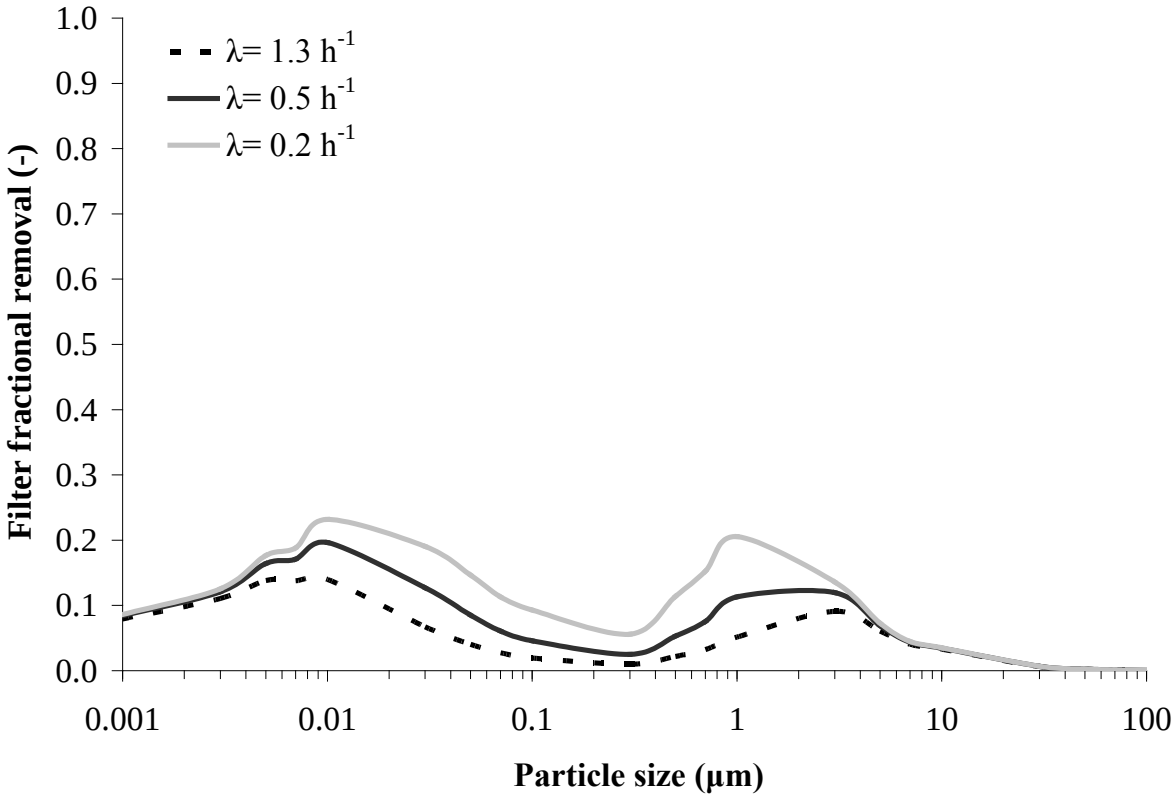


Figure 10. Filter capture probability curves for different air exchange rate scenarios.

This analysis suggests that HVAC filters may be used as passive samplers that are in place for long periods of time and can overcome the short-term sampling limitations of traditional air samplers. In particular, HVAC filters capture particles over a wide size range and can be considered effective overall samplers. In contrast, as can be seen in Figure 7, settled dust samples are biased toward larger particles, while conventional air samples may oversample those particle sizes that are not removed effectively by other mechanisms and tend to stay longer in the air (0.03 - 3 µm range). The best way to increase the probability that a broader size range of indoor particles will be captured by the HVAC filter is to increase the filter efficiency. This variable has a greater predicted effect than increasing the λ_r or decreasing the λ. High efficiency filters, in particular,

could develop into a less intrusive and effective way to obtain information regarding the indoor contamination in homes.

4.4 Evaluation of HVAC filters as samplers

During this dissertation, several aspects related to the use of filters were investigated and the findings contribute to assessing the potential use of HVAC filters as samplers. Filters could be a valuable sampling option that may be utilized as long-term samplers with minimal intrusion into homes and commercial buildings. This information could be integrated with conventional indoor air sampling strategies or, depending on the data being sought, it may provide an alternative, more efficient mechanism for collecting samples during large-scale investigations of multiple residences. While filters have the potential to be analyzed for a wide range of contaminants, this dissertation focused on microbial contaminants and, to a lesser degree, on heavy metals. The results indicate that filters can be used to assess bacterial and fungal concentrations and communities in residences. The filter samples yielded similar information to that obtained from samples collected in other indoor locations. During the field investigations, the importance of several confounding factors was observed including the influence of particle size, HVAC filter efficiency and HVAC system operation on the likelihood of filters to collect particles. These parameters were investigated in the last phase of the investigation where the model results indicated that HVAC filters can represent a valid sampling alternative for a wide range of particle sizes and conditions.

However, as with all sampling methodologies, using HVAC filters as samplers has limitations that must be considered when interpreting the data collected. For instance, the influence of filter location relative to potential particle generation sources is a variable that should be considered and investigated further. In an investigation related

to the effectiveness of portable air cleaners, Novoselac and Siegel (2009) reported the importance of device location with respect to the particle source. Similarly, we expect that the locations of the filter and return vent will be important factors that affect particle capture on the HVAC filter.

An additional limitation of using HVAC filters to sample the indoor environment is that in certain geographical regions or among specific socioeconomic groups, a significant fraction of the residences may not have a centralized air conditioning system, or the system is not used for certain seasons and, therefore, filters are not a sampling option. Even for the buildings that have a centralized air-conditioning system with built-in filtration, the occupants have significant control over several important factors including the efficiency of the installed filter and the duty cycle of the HVAC system. Filters have a possibility of collecting particles only when they are operated and, as a consequence, when there is limited need for conditioning, filters are unlikely to be effective samplers. Additionally, even if HVAC systems have elevated air recirculation rates, the use of filters as samplers will be affected by when the system is operated relative to when the contamination occurs and, therefore, the importance of HVAC system cycling may require further investigation. Filters located in systems with elevated return side leakages may not be representative samplers of indoor particle-bound contaminants, particularly if the ducts and system are located in the unconditioned space. Finally, another factor to be considered is how to obtain a representative sample of what is captured on the filter. This is an important aspect that requires a careful sampling procedure because certain particles may tend to stay attached to the filter fibers, leading to a bias based on the particles that are actually recovered and analyzed. In the current study, during Phase 1, filter dust samples were collected by shaking and scraping the dust off the filter in order to be able to obtain a concentration measurement. Possibly, some

particles stayed in the filter and this may have influenced the analysis. Similarly, if the filter dust sample was acquired by vacuum the dust from the filter, many particles could have stayed attached to the filter fibers. In Phase 2, since the goal was not to obtain a concentration but to investigate the microbial communities, nine 2.54 cm square pieces of filter material distributed in each quadrant were cut from the filter and the DNA from the microorganisms present on these pieces were directly extracted as illustrated in Appendices B and D.

5. SUMMARY AND CONCLUSIONS

The main contribution of this dissertation to the indoor air field is the evaluation of HVAC filters as a sampling mechanism for indoor contamination. To my knowledge, the relationship between the contaminants observed on HVAC filters and those observed in other indoor locations has not been explored in sufficient detail before to assess their potential application as samplers for residences. Specifically, this dissertation revealed that HVAC filters could be used to assess culturable microbial concentrations in buildings with levels similar to those observed in other indoor sampling locations and to the values reported in the literature. HVAC filter dust seems to be a favorable environment for microorganisms for the specific conditions present during this study (mostly warm and humid). The concentrations observed in different indoor sampling locations and across filters with varying efficiencies suggest that microbial concentrations are not likely to be influenced by particle size. Metal concentrations observed in the field investigation of residences revealed that Pb is present in higher levels than Cd and As, and may be associated with the age of the buildings suggesting a possible correlation with leaded-based paint. Metal concentrations in HVAC filter dust are statistically lower than that observed in high surface and floor dust samples. Additionally, dust samples from low efficiency filters had greater metal concentrations than did high efficiency filters. The last two points indicate that small particles may have greater metal concentrations than larger particles as previously suggested by other studies.

The investigation of culturable microorganisms revealed similarity in the concentrations in different indoor sampling locations. However, although the concentrations are similar, the composition of the microbial communities could still be different, emphasizing the importance of fully characterizing the microbial communities.

During this dissertation, I addressed this aspect and the comparison of bacterial and fungal communities revealed statistically similar compositions between dust samples collected from high efficiency HVAC filters and high surfaces in residences. Additionally, the findings from a detailed investigation in an unoccupied test house support the use of HVAC filters as surrogate for long-term air samples. Proteobacteria were observed in greater proportion on HVAC filter dust and in air samples suggesting the air origin of this phylum. In contrast, Firmicutes and Actinobacteria were detected in greater proportion in residences than in the unoccupied test house supporting the speculations that these phyla are associated with occupants.

During Phase I and II, a variety of factors played a critical role in the use of HVAC filters as samplers. Therefore, in Phase III, a modeling approach was utilized to evaluate the removal of indoor airborne particles via different removal mechanisms and to assess the likelihood that filters will be effective samplers. The application of the model to typical residential scenarios revealed that deposition is the dominant removal mechanism for large and small particles and, as a consequence, surface dust samples may be biased toward larger particles. In contrast, exfiltration is the dominant mechanism for particles not effectively removed by other mechanisms, and air samplers may overemphasize particles with elevated air residence time. Filter efficiency and the air recirculation rate through the HVAC system play an important role in the application of filters as samplers. High efficiency filters placed in systems intensively operated are likely to collect a greater fraction of particles across a wide size range. In contrast, the air exchange rate of the building has little impact and, therefore, HVAC filters could be used as samplers almost independently of the tightness house.

This dissertation provides evidence that HVAC filters can be considered a valid sampling option for particle-bound contaminants. The filters can be easily collected and

analyzed for a wide range of contaminants. Additionally, filters are in place for extended periods of time and can be used as surrogate for long-term air samples without some of the limitations associated with short-term air samples. Nevertheless, additional studies are required to investigate other important issues associated with the application of filters as samplers including the location of filters relative to the contaminant source, the zoning of the residences, and the influence of resuspension of deposited particles. Additionally, depending on the nature of the contaminants of interest, specific aspects could be important. For instance, for metals the size of the particles associated with contaminants seems to be a critical parameter. For microbial community studies, an important factor that deserves further investigation is the influence that different environmental conditions present on filters versus those present indoors may have on the survivability and growth of different microbial species. In particular, the importance that nutrient levels and relative humidity conditions have on the proliferation of certain species on filters relative to those present in the indoor core space should be investigated. Finally, since filter samples can be considered an integrated (long-term) measurement, the contaminant concentrations could ideally be linked to the predicted indoor contaminant concentrations in order to obtain estimates of occupant exposure during the time the filters were in place.

Appendix A

PAPER I

BIOLOGICAL AND METAL CONTAMINANTS IN HVAC FILTER DUST

(Published in ASHRAE Transactions. 2009. 115 (2), 484-491)

ABSTRACT

Recently, the interaction between particles retained on HVAC filters and indoor air quality has gained more attention due to their possible relationship to irritation, health outcomes, and odors. This paper focuses on microbial contaminants and metals captured on HVAC filters in nine residential and light-commercial buildings. Culturable fungi and bacteria populations captured in the dust were quantified using standard spread plate methods and heavy metal (Pb, As, Cd) concentrations were determined by atomic absorption spectroscopy. Culturable fungal and fungal spore concentrations ranged from 10^4 to 10^6 and from 10^2 to 10^3 CFU/g, respectively, while culturable bacteria and bacterial spore concentrations ranged from $10^5 – 10^7$ and $10^3 – 10^5$ CFU/g, respectively. Microbial concentrations were consistent across filters having different efficiencies with median concentrations within one order of magnitude. Heavy metal concentrations were as high as 29 μg/g for lead, 6 μg/g for cadmium, and 7 μg/g for arsenic. Variations observed in the metal concentrations between different dust samples may be due to particle size differences related to different filter efficiencies and indoor sources. This investigation provides insight into possible metal sources and concentrations of biological and heavy metal contaminants present in indoor environments.

INTRODUCTION

Indoor air quality researchers typically focus their attention on biological, chemical and particulate contamination of indoor environments and the health effects and

discomfort that these contaminants may cause. Indoor environmental investigations typically rely on short-term sampling techniques that provide only a snapshot of contaminant concentrations in the indoor environment at the time of sampling. HVAC filter dust is a potential resource that has received less attention and may enhance our understanding of indoor occupant exposure. Filters are typically in place for extended periods of time and have the potential to serve as long-term samplers of the indoor environment. Furthermore, HVAC filter dust can be collected with minimal effort and analyzed for a broad range of contaminants. This paper focuses on bacteria, fungi, and heavy metals captured on HVAC filters and investigates how these parameters vary with filter and building characteristics.

Several studies have measured the concentration of bacteria and fungi in indoor environments, especially in air and settled dust (e.g., Bouillard *et al.*, 2005; Dales *et al.*, 1997; Verhoeff and Burge, 1997). However, the reported concentrations are difficult to compare because they vary considerably depending on sampling technique and sampling location, among other factors. An alternative approach for investigating air and settled dust would be to analyze the dust that collects on HVAC filters. A recent study has suggested that HVAC dust may provide an integrated measure of airborne contamination levels in an indoor environment (Tringe *et al.*, 2008). HVAC filters are able to retain biological particles and microorganisms can survive, accumulate, and, under certain conditions, multiply on HVAC filters (Farnsworth *et al.*, 2006; Foarde and Hanley, 2001; Kemp *et al.*, 1995; Kemp *et al.*, 2001; Moritz *et al.*, 2001; Simmons and Crow, 1995). In addition, a number of studies suggest a relationship between Sick Building Syndrome (SBS) symptoms and the presence of microorganisms on filters (e.g., Schleibinger and Ruden, 1999). Several researchers have also studied heavy metal concentrations in house dust and the correlation with potential indoor and outdoor sources and particle size

distributions (Al-Rajhi *et al.*, 1996; Chattopadhyay *et al.*, 2003; Decker *et al.*, 2002; Kim *et al.*, 1998; Tong, 1998). Despite these efforts, we are not aware of any research that utilizes HVAC filters as samplers to characterize metal concentration levels indoors or that examined the influence of HVAC systems and potential sources on metal concentrations found on the HVAC dust.

While both microbial populations and metals found indoors have been studied, the relationship between their presence in HVAC filter dust and critical characteristics of both the particular HVAC system and the building remains unclear. This research compares the contaminant levels found in HVAC filters with different filter efficiencies and provides insight into potential sources of contamination. This investigation is part of a broader evaluation of the utility of using filters as samplers for the indoor environment.

METHODOLOGY

Eight residential and one commercial building in Austin, Texas were selected for this investigation. These sites represent a sample of convenience and not a random sample. To characterize the sites considered, data was collected regarding the year the buildings were built, number of occupants, past or current presence of smokers, proximity to major highways, presence of attached garage, filter location, and conditioned volume. Two sets of HVAC filters were collected from each site, approximately three months apart. All filters were stored in a 4º C (39 ºF) environmental chamber maintained at a relative humidity (RH) of approximately 70% until the analyses were performed within a few weeks following collection.

Characterization of Sites and Filters

The filters were categorized according to the minimum efficiency reporting value (MERV) as determined by ASHRAE Standard 52.2 (ASHRAE, 2007) and reported by

the manufacturers. The sample included seven low-efficiency filters (MERV <5), seven mid-efficiency filters (MERV 5-8) and four high-efficiency filters (MERV 9-14). Filter pressure drop measurements were performed at filter installation and removal using an Energy Conservatory DG700 digital manometer, and the mean value of these two measurements characterized the mean filter pressure drop. Mean flow rates across each filter in fan-only mode were measured using an Energy Conservatory True Flow Plate. By monitoring the HVAC systems two or three times during the cooling season for 24 hours approximately every month, we measured the cooling duty cycle, which is an estimate of the fraction of time that the HVAC system is running during the cooling season. In addition, during the monitoring events, the temperature and RH in the HVAC system return plenum were also recorded. To estimate the mass accumulated on each filter, we subtracted the mean weight of three unused filters from the weight of the used filter using a balance (Sartorius B310S). Table 1 summarizes the instruments used during the investigation.

Table 1: Summary of Instrumentation

Measurement	Manufacturer	Model	Accuracy
Temperature	Onset	Hobo U10	±0.4 °C (0.7 °F)
Relative humidity	Onset	Hobo U10	±3.5%
Pressure drop	Energy Conservatory	DG 700	±1% or 0.2 Pa (0.0008 IWC)
Air flow	Energy Conservatory	True Flow Plate	±7%
Weight	Sartorius	Balance B310S	±0.001 g

Microbial and Metal Analyses

Two samples of dust from each filter were acquired by shaking and scraping the dust material off the filters. The samples were subsequently analyzed for microbial and heavy metal concentrations. The enumeration of culturable bacteria and fungi was completed using the standard spread plate method 9215C (APHA, 1998). The

microorganisms present in the HVAC filter dust were transferred into a phosphate buffer solution (PBS, 8 g/L NaCl, 0.2 g/L KCl, 1.44 g/L Na_2HPO_4, and 0.24 g/L KH_2PO_4) by sonication and vortexing for 10 minutes each. For bacterial enumeration, a 0.1 ml aliquot of PBS was plated on R2A agar plates containing 0.04% cycloheximide. For fungal determinations, a 0.1 ml aliquot of PBS was plated on Sabouraud Dextrose Agar (SDA) plates containing 0.01% chloramphenicol. Bacterial plates were incubated for 3-7 days at 30 °C (86 ºF), while fungal plates were incubated for 7-14 days at room temperature (approximately 23 °C/73 ºF). After incubation, the number of bacterial and fungal colonies formed was counted and the results were used to estimate the microbial concentration in the dust, expressed as colony forming unit (CFU) g^{-1} dust. The analysis was performed three times for each dilution and the average number of colonies formed was recorded. The ability of the microorganisms to form spores was also tested by pasteurizing an aliquot of the samples for 15 minutes at 75º C (167 ºF) and then plating the samples as described above. Any colonies that formed were assumed to have originated from spores and to represent the spore-forming fraction of the population.

Heavy metal concentrations in the HVAC filter dust were determined by atomic absorption spectroscopy (PerkinElmer AAnalyst 600). Dust samples were digested via the microwave-assisted digestion method 3030K (APHA, 1998). This method consists of a nitric acid digestion under controlled pressure and temperature conditions that facilitate the transfer of the metals present in the particles into the liquid extract. The liquid extract from each sample was analyzed for selected heavy metals (Pb, As, Cd) according to method 3111B (APHA, 1998). To ensure the accuracy of the measurements, reagent blanks and periodic calibration checks were also analyzed.

A nonparametric statistical method, the Wilcoxon Rank-Sum Test, which does not assume any specific distribution of the data, was applied to compare and identify

dissimilarities between the different data groups. When comparing the different data groups, a significance level of 0.1 was assumed owing to the small sample size and the conservative nature of this statistical test.

RESULTS AND DISCUSSION

Table 2 characterizes the nine sites and the presence of likely sources of contamination. Site 9 was the one light commercial building included in the study. All the sites were relatively close to major highways and five sites had attached garages. Sites 2, 3, 4, and 9 were attached to other dwellings. Four sites had the filter located at the unit, while five were located at the return register. In two of the Sites (3 and 9), multiple filters were present. Cooling duty cycles of the sites ranged from 9 to 34%. There were no smokers occupying any of the sites, although Site 2 had had smokers in the past. Sites 3 and 4 were located in the same residence with two separate and independent HVAC systems for different floors of the residence. The sites summarized in Table 2 represent a range of HVAC systems and operating characteristics for this region of the country.

Table 2: Site Characteristics

Site #	Year built	# of occupants	Proximity to highway [km (miles)]	Attached garage	Carpet	Filter location	Conditioned Volume [m^3 (ft^3)]	Cooling duty cycle [%]
1	1975	2	1.0 (0.62)	Yes	No	Unit	422 (14,900)	14
2	1973	2	0.6 (0.37)	Yes	Yes	Unit	309 (10,900)	16
3	1998	1	0.2 (0.12)	Yes	No	Register2	114 (4,020)	9
4	1998	1	0.2 (0.12)	Yes	Yes	Register	227 (8,010)	27
5	1949	2	1.8 (1.12)	No	No	Register	276 (9,740)	32
6	1941	4	1.1 (0.68)	No	Yes	Register	324 (11,400)	29
7	Late 70s[1]	4	0.6 (0.37)	No	Yes	Unit	259 (9,140)	34
8	1984	3	0.5 (0.31)	Yes	Yes	Unit	308 (10,900)	15
9	1995	3	0.2 (0.12)	No	Yes	Register3	656 (23,200)	19

[1]Estimated based on neighborhood and nearby homes.
[2]Three filters in different return grilles were present at this site

[3]Two filters in different return grilles were present at this site

Table 3 summarizes the characteristics of the 18 HVAC filters, two from each site, that were evaluated during the project. The mean pressure drop across the filter, ΔP, and the mean volumetric airflow through the HVAC system, Q, were obtained by averaging the values obtained at installation and at removal. For a few filters, we were not able to measure the filter pressure and supply plenum pressure (required for the flow measurement) at filter installation, so the measurements collected at the time of filter removal are reported. For Filter 2 of Site 2, the value reported represents the observation acquired at installation. The mean temperature and RH observed at the HVAC return plenum during the monitoring events are also reported. These values do not represents mean levels during the period the filters were in place, but only what was observed during the monitoring visits.

For some filters, the days in service was not known, because it was the filter the homeowner had in place when we started the investigation. For seven filters it was possible to estimate the mass accumulated over the service life because these filters were weighed before use. As expected, we observed a correlation between filter efficiency and particle mass accumulated on the filter. The mean mass accumulated on the low-efficiency and mid-efficiency filters was 1.7 and 4.0 g, respectively. There may also be a correlation between the mass of particles accumulated on filters and the presence of carpet in the house. The mean mass accumulated on the filters from the sites with and without carpet was 3.9 and 0.8 g, respectively. Carpets tend to accumulate more dust than bare floors because they are harder to clean than other types of floor. As a consequence, particle resuspension from carpet is expected to be greater than from other floor surfaces (Yoon and Brimblecombe, 2000). As demonstrated by Corsi *et al.* (2008), resuspension

of PM_{10} is much larger than $PM_{2.5}$ suggesting that even the low MERV filters can retain many of the larger particles from vacuuming activities.

Table 3: Filter Characteristics

Site	Filter	Filter Efficiency	Pressure Drop, ΔP [Pa (IWC)]	Air flow, Q [m^3/h (cfm)]	Temperature [°C (°F)]	RH [%]	Days in service	Mass on filter [g]
1	1	Low	22 ± 0.2 (.088 ± 0.0008)	1710 ± 120 (1010 ± 71)	24.7 ± 0.50 (76.5 ± 33)	70.8 ± 4.1	88	
	2	Mid	50 ± 0.5 (.20 ± 0.002)	1670 ± 120 (981 ± 69)			95	
2	1	Low		1280 ± 90 (754 ± 53)				
	2	Low	58 ± 0.6 (.23 ± 0.002)	1780 ± 130 (1050 ± 74)1	26.6 ± 0.83 (79.8 ± 34)	66.7 ± 3.7	95	4.5 ± 0.002
3	1	High	37 ± 0.4 (.15 ± 0.002)	1420 ± 100 (837 ± 59)	25.5 ± 0.77 (78.0 ± 33)	62.4 ± 4.5	85	
	2	Mid	33 ± 0.3 (.13 ± 0.001)	1450 ± 100 (851 ± 59)			99	1.3 ± 0.002
4	1	Low	64 ± 0.62 (.26 ± 0.002)	941 ± 66 (554 ± 39)2				0.3 ± 0.002
	2	High	54 ± 0.5 (.22 ± 0.002)	1000 ± 70 (589 ± 41)	26.1 ± 0.87 (78.9 ± 34)	58.4 ± 3.5	85	
5	1	Mid	78 ± 0.82 (.31 ± 0.003)	1990 ± 140 (1170 ± 82)				
	2	Low	59 ± 0.6 (.24 ± 0.002)	1940 ± 140 (1140 ± 80)	24.4 ± 1.9 (75.9 ± 35)	63.0 ± 5.9	87	0.3 ± 0.002
6	1	High	89 ± 0.92 (.36 ± 0.004)	1800 ± 130 (1060 ± 74)2				
	2	Mid	92 ± 0.9 (.37 ± 0.004)	1660 ± 120 (975 ± 68)	24.8 ± 1.3 (76.6 ± 34)	58.9 ± 5.0	90	4.2 ± 0.002

7	1	Low	49 ± 0.5 (.20 ± 0.002)	1300 ± 91 (763 ± 54)	26.0 ± 0.30 (78.8 ± 33)	60.1 ± 2.9	87	
	2	Mid	81 ± 0.8 (.32 ± 0.003)	1140 ± 79 (669 ± 46)			92	4.1 ± 0.002
8	1	Mid	48 ± 0.52 (.19 ± 0.002)	1150 ± 80 (676 ± 47)2				
	2	Low	27 ± 0.3 (.11 ± 0.001)	1200 ± 84 (705 ± 49)	24.7 ± 1.3 (76.5 ± 34)	52.5 ± 3.9	88	
9	1	High	76 ± 0.82 (.30 ± 0.003)	2730 ± 190 (1610 ± 110)				
	2	Mid	81 ± 0.8 (.32 ± 0.003)	2790 ± 200 (1640 ± 120)	24.0 ± 1.6 (75.2 ± 35)	54.2 ± 5.3	82	6.5 ± 0.002

[1]only initial measurement
[2]only final measurement

Figure 1 presents the mean culturable microbial concentrations in the HVAC filter dust from the nine sites investigated, expressed as CFU/g dust. Since two filters were collected and analyzed from each site, 18 total samples are represented in Figure 1 and the mean value for each site is shown. For each site, the left bar indicates the culturable concentration of bacteria while the right bar represents the culturable fungal concentration. The height of each bar indicates the mean culturable concentration and originated from the counts of the microbes with the ability to form colonies on the specific agar plates described in the Methodology section. The bottom section of each bar represents the spore forming fraction of the population, which is the fraction of the viable microbial concentration able to survive the pasteurization treatment. Only the error bars for the total height of the columns are shown in the figure and the bars on the lower portions were of similar magnitude.

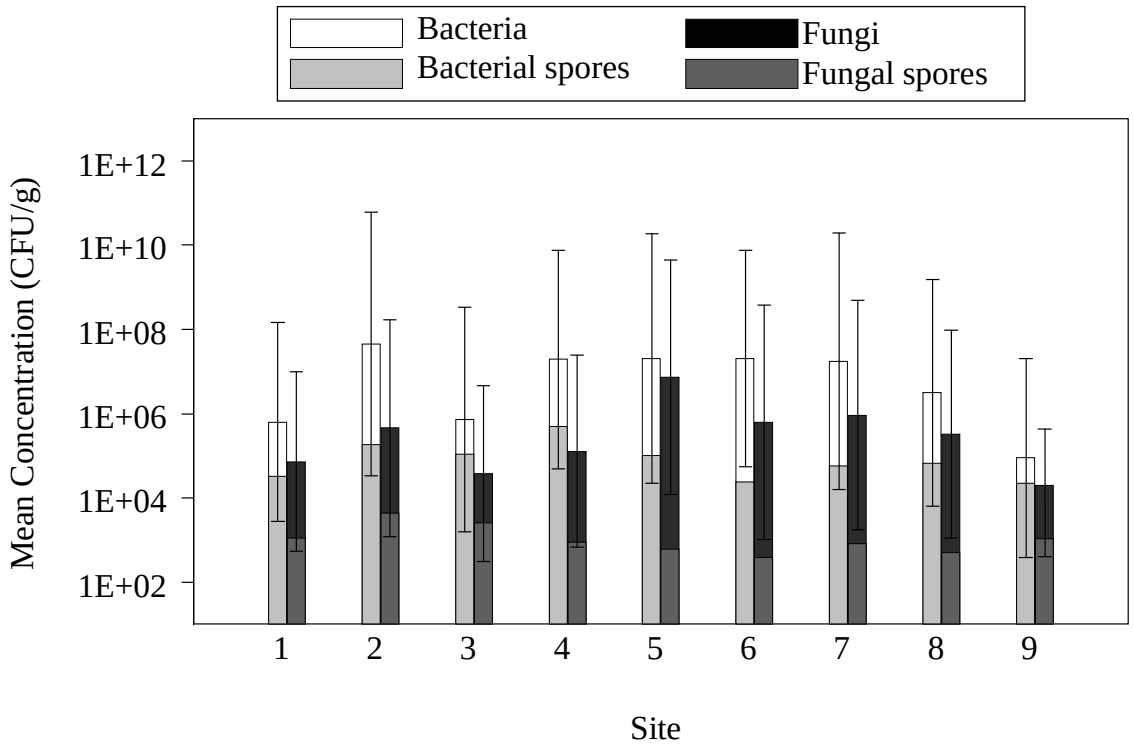


Figure 1. Mean microbial concentrations in HVAC filter dust.

The culturable bacterial concentrations were consistently greater than the fungal concentrations for the nine sites investigated. The bacterial concentrations ranged from 10^5 to 10^7 CFU/g while the bacterial spore concentrations were typically two orders of magnitude lower, ranging from 10^3 to 10^5 CFU/g. The mean concentration across the sites was 1.4×10^7 CFU/g for bacteria and 1.2×10^5 CFU/g for bacteria spores. Culturable fungal concentrations were consistently lower than bacteria levels and varied in the 10^4 - 10^6 CFU/g range. Fungal spore concentrations were typically the lowest of all four categories and varied in the 10^2 - 10^3 CFU/g range. The mean concentration across the sites for fungi and fungal spores was 1.1×10^6 and 1.4×10^3 CFU/g, respectively. To put

these microbial concentrations in context, these values are similar to those observed in soil for both bacteria and fungi (Lovell *et al.*, 1995; Toro *et al.*, 1997).

The culturable bacterial and fungal concentrations observed in the current study are slightly higher than the values reported in the literature for settled dust (Bouillard *et al.*, 2005; Chew *et al.*, 2003). This difference may be attributable to the HVAC airflows that deliver airborne microbes and nutrients to HVAC filters. Many studies have suggested that microbial contamination of HVAC filters occurs because filters collect sufficient organic material and nutrients to support microbial growth (Burge, 1987; Kemp *et al.*, 2001; Pejtersen, 1996). Kemp *et al.* (1995) also observed enhanced fungal growth when additional nutrients were delivered to HVAC filters. The culturable microbial concentrations encountered in this study suggest that HVAC filters in residential buildings in a humid environment like central Texas during the cooling season represent a hospitable environment for microbial proliferation.

The microbial concentrations measured in this study represent only the culturable fraction of the microbial population able to grow on the specific media utilized. Toivola *et al.* (2002) estimated that only 1% of the microbial population indoors is culturable and molecular based tools offer the promise of being able to detect a much greater fraction of the microbial community, not just the culturable fraction. However, the extraction of DNA directly from HVAC filter dust cake is particularly challenging and, as reported by Ramakrishnan *et al.* (2006), the use of standard commercial DNA extraction kits often generates inconsistent results. Nevertheless, the authors are currently investigating these techniques and their applicability to further characterize microbial populations on HVAC filters.

Table 4 summarizes the median microbial concentrations observed on filters with different MERV ratings. Median microbial concentrations on HVAC filters were

relatively consistent across filters with different removal efficiencies. The median concentrations were typically within one order of magnitude of each other and application of the Wilcoxon Rank-Sum Test to the data did not find any significant differences between filters with different MERV ratings. Despite this general similarity, high-efficiency filters had the lowest median microbial concentrations for bacteria, fungi and fungal spores. As reported by Waring and Siegel (2008), the particle mass that accumulates on HVAC filters strongly depends on their removal efficiency, and high-efficiency filters capture a greater mass of particles. Typical bacteria and fungi cell sizes vary from less than a micron to several microns, depending on the microbial species. Therefore, high-efficiency filters are more likely to retain an elevated number of microbial cells. A high-efficiency filter also captures more non-biological particles, potentially providing microorganisms with a greater amount of substrate and nutrients, and therefore promoting their growth. However, the presence of non-biological particles will also increase the mass captured on the filters and serve to diminish the measured microbial concentration because it is based on CFU per unit mass (both biotic and abiotic) of dust captured. This is one possible explanation for the decreased microbial concentrations observed on the dust captured in the high-efficiency filters.

Table 4: Median microbial concentrations in HVAC filter dust for filters with different efficiencies.

Filter MERV	Bacteria	Bacterial spores	Fungi	Fungal spores
	CFU/g			
Low	$6\times10^{6} \pm 6\times10^{5}$	$5\times10^{4} \pm 9\times10^{3}$	$4\times10^{5} \pm 7\times10^{4}$	$1\times10^{3} \pm 1\times10^{3}$
Mid	$9\times10^{5} \pm 2\times10^{5}$	$7\times10^{4} \pm 7\times10^{3}$	$6\times10^{5} \pm 1\times10^{5}$	$8\times10^{2} \pm 1\times10^{3}$
High	$3\times10^{5} \pm 6\times10^{4}$	$7\times10^{4} \pm 3\times10^{3}$	$1\times10^{5} \pm 9\times10^{4}$	$6\times10^{2} \pm 7\times10^{2}$

Figure 2 summarizes the mean HVAC filter dust concentrations of lead, cadmium and arsenic for each site. Pb had consistently the greatest concentration in all the samples

with values ranging from 5.4 to 28.6 µg/g dust. The median Pb concentration across all samples was 13.0 µg/g. HVAC filter dust concentrations for Cd and As were lower than Pb concentrations with values varying in the 0.5 - 6 and 0.8 - 7.3 µg/g ranges, respectively. The median concentrations of Cd and As across all the samples analyzed were 1.9 µg/g and 1.4 µg/g, respectively. The metal concentrations reported in the literature for indoor dust are similar to those reported here for HVAC filter dust and are typically in the µg/g range, with Pb and Zn concentrations that tend to be higher than the other metals and can reach the mg/g range (Al-Rajhi *et al.*, 1996; Lisiewicz *et al.*, 2000; Turner *et al.*, 2006).

Sites 5, 6 and 7 had higher Pb concentrations than the rest of the sample. None of the three sites had attached garages or is located adjacent to a major highway, suggesting that leaded gasoline is not the major source of indoor lead. Sites 5 and 6 were the oldest sites investigated and we hypothesize that the elevated Pb concentration was derived from leaded paint, still in use when the residences were built. Several researchers (Chattopadhyay *et al.*, 2003; Kim *et al.*, 1998; Tong, 1998) provide evidence for this hypothesis. There was uncertainty about the age of Site 7, the other site with an elevated Pb concentration although it was located in a neighborhood constructed in the 1970s and was likely to have contained leaded paint. Site 3, the newest residence investigated had the lowest Pb concentration again supporting the hypothesis that leaded paint is an important contributor to indoor lead levels. A correlation between the age of a property and Pb levels in settled dust has also been observed by other researchers (Adgate *et al.*, 1998; Kim *et al.*, 1998; Tong, 1998). However this correlation is not entirely consistent throughout our study; for instance, Site 4, which is also a new residence, had a higher Pb concentration than several older sites in the study so other factors may be important. At a given site, our data suggests that a correlation between the Pb, Cd and As metal

concentrations may exist, as suggested by Sites 5, 6, and 7. In these sites, the concentrations of the three metals analyzed are all above the median values observed in this study suggesting a common source, or coincident sources, of metal contamination.

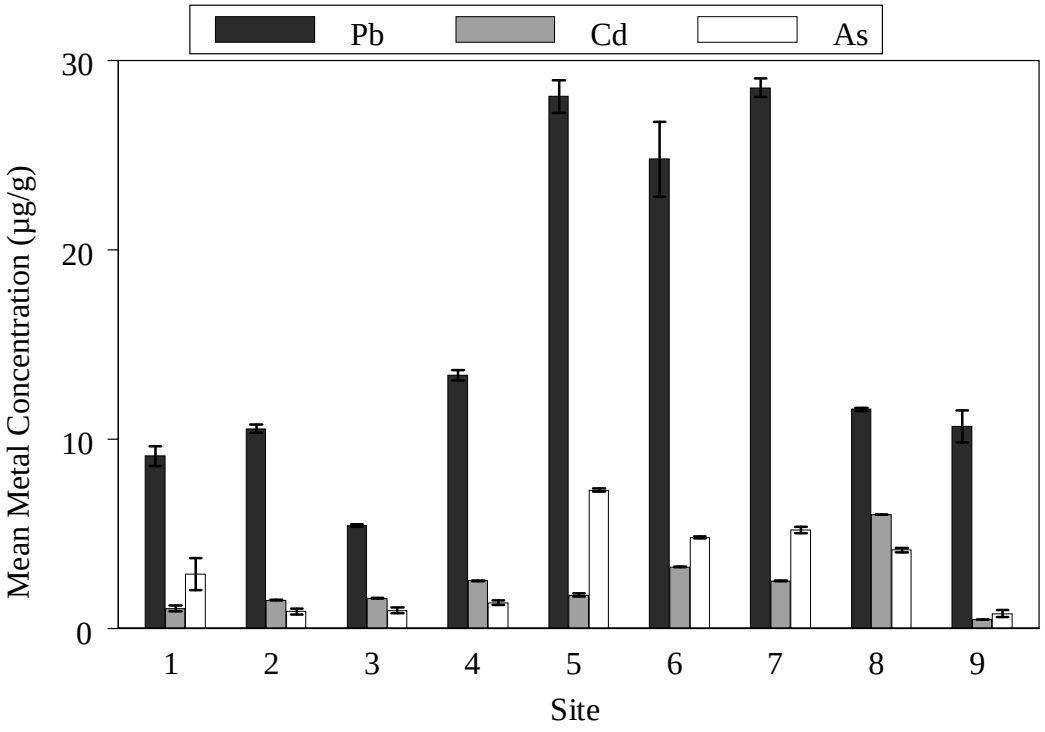


Figure 2. Metal concentrations in HVAC filter dust.

Table 5 summarizes the median concentrations of Pb, Cd and As in HVAC filter dust collected on filters with different removal efficiencies. The median metal concentrations for the high-efficiency and low-efficiency filters were always the lowest and the greatest, respectively. For Pb and, especially, As, the concentrations in the high-efficiency filters were significantly lower than those in the low-efficiency filters. As described above, high-efficiency filters retain a greater fraction of small particles than low-efficiency filters. Low-efficiency filter dust has a greater proportion of larger particles than high-efficiency filter dust. Therefore, the data suggest that large particle

size fractions may have greater metal concentrations than small particle size fractions. This observation is in accordance with the findings of Al-Rajhi *et al.* (1996). However, another study (Lisiewicz *et al.*, 2000) detected higher metal concentrations in fine particles than in larger particles. General conclusions are difficult to draw because of the limited number of sites investigated in the current study and because the filters with different efficiencies were not uniformly distributed throughout the sites and, therefore, some biases due to potential site-specific sources are possible. Furthermore, different metal sources, both indoor and outdoor, may have a significant influence on the metal concentration distribution for particles of different sizes. In our study, Cd concentrations were extremely uniform across filters with different efficiencies and the Cd concentrations detected were comparable to values reported in the literature for settled dust (Jaradat *et al.*, 2004; Momani *et al.*, 2002; Turner *et al.*, 2006). The concentration of As in the low-efficiency filters was greater than those for the mid- and high-efficiency filters, suggesting that the As concentrations in the larger particle size fractions could be particularly elevated. Decker *et al.* (2002) associated elevated indoor dust levels of arsenic with pressure-treated wood.

Table 5: Median metal concentrations in the HVAC filter dust for filters with different efficiencies.

Filter MERV	Pb	Cd	As
		µg/g	
Low	18.5 ± 0.79	2.00 ± 0.062	4.61 ± 0.24
Mid	12.9 ± 0.54	1.64 ± 0.035	1.89 ± 0.32
High	7.49 ± 0.44	1.54 ± 0.027	0.912 ± 0.22

Table 6 summarizes the mass of metals collected on the HVAC filters, calculated as the metal concentration multiplied by the mass of dust collected on the seven filters where the dust mass was measured. Filter 2 at Site 6 appears to be highly contaminated

with all three metals. Metal mass seems to have similar trends for the three metals and it is unusual for a site to have a low concentration of one element and an elevated concentration of another one. Table 7 summarizes the median metal mass on filters with low and mid efficiencies. Mid-efficiency filters collected a higher mass of all three metals analyzed. Filter efficiency data obtained from ASHRAE Standard 52.2 (ASHRAE, 2007) indicates that mid-efficiency filters capture approximately two to three times as many 3.0 – 10.0 µm particles than the low-efficiency filters. Similar (or even stronger) trends in capture efficiency are also expected for smaller particles and, this difference in removal efficiency could explain the different quantities of metals retained on the filters.

Table 6: Metal mass on HVAC filters

Site	Filter	Filter Efficiency	Pb	Cd	As
			Amount of metal (µg)		
2	2	Low	11.8 ± 1.6	9.09 ± 0.69	2.30 ± 2.4
3	2	Mid	3.48 ± 0.18	0.882 ± 0.045	1.57 ± 0.66
4	1	Low	5.11 ± 0.28	0.752 ± 0.033	0.407 ± 0.032
5	2	Low	8.51 ± 0.81	0.470 ± 0.079	3.18 ± 0.14
6	2	Mid	192 ± 33	22.6 ± 1.1	37.4 ± 2.0
7	2	Mid	53.0 ± 0.92	3.80 ± 0.064	14.5 ± 0.29
9	2	Mid	36.7 ± 8.7	1.51 ± 0.031	3.47 ± 0.35

Table 7: Median metal mass on HVAC filters with different efficiencies.

Filter MERV	Pb	Cd	As
	Amount of metal (µg)		
Low	8.51 ± 0.81	0.752 ± 0.079	2.30 ± 0.14
Mid	44.9 ± 4.8	2.66 ± 0.055	8.96 ± 0.51

There are several parameters that may play a significant role in the application of HVAC filters as samplers and could potentially represent confounding factors in data interpretation. Further investigation is required to understand the influence of size-resolved filter efficiency, indoor mixing conditions, HVAC system run time, microbial growth and decay in filter dust, and particle-size dependence of the contaminants of

interest. Once the impact of these factors is better delineated, HVAC filters may become a useful, widely-available sampling tool that can be collected with minimal effort and analyzed for a broad spectrum of contaminants.

CONCLUSIONS

We measured microbial and metal concentrations in HVAC filter dust collected from nine sites. We detected culturable bacterial and fungal concentrations in the 10^5 - 10^7 and 10^4 - 10^6 CFU/g ranges, respectively. Spore concentrations represented a smaller fraction, typically two to three orders of magnitude lower than the total concentrations. The microbial concentrations in the filter dust were slightly higher than settled dust concentrations reported in the literature and are in the same range as those reported for soil. These results indicate that HVAC filters in humid environments such as central Texas represent a hospitable environment for microbial proliferation. Microbial concentrations on filters with different removal efficiencies were relatively similar, typically within one order of magnitude. Mean Pb concentrations in the HVAC filter dust were particularly elevated with mean values as high as 29 µg/g, while Cd and As concentrations were on the order of a few µg/g. A possible correlation between the age of the site and the Pb concentration was observed suggesting that leaded paint is a possible source of indoor Pb dust. Differences in heavy metal concentrations were observed between buildings and filters, suggesting that several factors including the influence of filter efficiency, system run time and indoor contaminant distribution need more exploration before filters can be used as sampling devices.

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Appendix B

PAPER II

EVALUATION OF HVAC FILTERS AS A SAMPLING MECHANISM FOR INDOOR MICROBIAL COMMUNITIES

(In preparation for submission to Indoor Air)

ABSTRACT

HVAC filters are in place for extended periods of time and can serve as integrated air samplers. This paper presents a comparison of bacterial and fungal concentrations and communities in HVAC filter dust and other sampling locations in occupied residences and in an unoccupied test house. A DNA-based, culture-independent approach was utilized to characterize the microbial communities. Microbial concentrations and communities in HVAC filter dust samples were not statistically different from those in high surface dust samples in occupied residences suggesting that filters could be used as samplers in buildings providing statistically similar information. Despite the general similarity in the communities, Proteobacteria were present in greater proportion in HVAC filter dust samples than on surface dust samples suggesting the air origin of this phylum. Gram-positive bacteria were present in greater proportion in occupied residences than in the unoccupied test house, confirming the potential association of this group with occupants. HVAC filter microbial communities were similar to those present in a composite indoor air sample providing preliminary evidence that filters could be a viable option for long-term investigation of airborne biological contaminants.

INTRODUCTION

The presence of microorganisms indoors has been related to several health and discomfort outcomes including respiratory diseases, odors, and occupant dissatisfaction (Gyntelberg et al, 1994; Verhoeff and Burge, 1997). Some researchers have associated indoor microbial concentrations with asthma symptoms (Park et al., 2006; Ross et al.,

2000; Smedje et al., 1997). However, the association between culturable fungal levels in air or dust samples and health problems has been inconsistent (Nelson et al., 1995; Peat et al., 1998; Verhoeff and Burge, 1997). This discrepancy may be attributable to the fact that bioaerosol samples are typically short-term in nature and provide only a snapshot of microbial contaminant levels in air at a particular time and place. Even when collected from the same location, airborne bacterial samples have significant temporal variability (Fierer et al., 2008) highlighting the need to develop an integrative methodology to assess indoor biological contaminants. Floor dust may provide an integrated sample of contaminant levels but these samples are influenced by material tracked-in from the outside and may be skewed toward larger particle-bound contaminants.

The majority of previous indoor biological studies have relied on an assessment of culturable microorganisms that represent only a small fraction of the total microorganisms present indoors (Toivola et al., 2002). In recent years, several studies have applied culture-independent, DNA-based approaches to better characterize the diverse bacterial and fungal communities present in indoor environments (Kelley et al. 2004; Pakarinen et al., 2008; Pitkäranta et al., 2008; Rintala et al., 2008; Täubel et al., 2009; Tringe et al., 2008). The application of molecular biology tools to indoor environmental investigations should reveal a much greater fraction of the microbial community present than culturable methods, a finding recently confirmed by Pitkäranta et al. (2008). Vesper et al. (2007) reported an association between asthma symptoms and the Relative Moldiness Index (RMI), an index based on molecular biology tools, confirming that these techniques may provide a better characterization of human exposure to microorganisms.

A potential alternative to the use of settled dust and air samples for microbial evaluation is the use of HVAC filters for indoor environment investigations. Collecting samples of HVAC dust may improve our understanding of indoor occupant exposure by providing an integrated measure of pollutant concentrations associated with indoor particles. Greater than 70% of the residential buildings in the United States have a central, forced HVAC system (US Bureau of Census, 2005), almost all with a built-in filtration system. These filters essentially serve as passive, long-term samplers that can be collected with minimal effort and analyzed for a broad range of indoor contaminants. Recently, Stanley *et al.* (2008) utilized filters in two large public buildings as bioaerosol sampling devices to determine the culturable bacteria concentrations and to identify selected culturable species present in air. While most of the molecular-based studies described above focused solely on settled dust, Tringe *et al.* (2008) investigated the microbial communities present on the dust that collected on two HVAC filters in two large shopping centers in Singapore. They reported that the two air samples (HVAC filters) have more in common to each other than with environmental samples (outdoor soil and water) collected in the proximity and originate from indoor niche. They also found more similarity between filter samples and indoor floor dust compared to outdoor ground-level dust. The purpose of the current study is to explore the microbial concentrations and communities on filters and compare them to indoor settled dust and air communities as a first step towards using HVAC filter dust as an integrated measure of microbial levels in residences. This paper focuses on bacterial and fungal culturable concentrations and communities present in HVAC filter dust and other indoor sampling locations in occupied residences and in an unoccupied full-scale test house. The investigation was divided into two phases: 1) Investigation of culturable microbial concentrations in settled and HVAC filter dust in eight occupied residences in Austin,

Texas; 2) Study of microbial communities, using a culture-independent, approach capable of revealing a much greater range of microorganisms, in four sites as well as on a full scale test house where a more detailed sampling was performed.

METHODS

Phase 1: Culturable microorganisms

A sample of convenience of eight residential buildings located in Austin, Texas was selected for this part of the investigation. All of the buildings had central air conditioning that recirculated indoor air, as is typical of residences in the Southern U.S.

Sample Collection

Floor dust, high surface dust and HVAC filter dust samples were collected 2-3 times over a six-month period from each residence during the cooling season (summer and fall). The sites investigated represent a sample of convenience and the filters were classified according to the minimum efficiency reporting value (MERV) as determined by ASHRAE Standard 52.2 (ASHRAE, 2007). The sample included seven low-efficiency (MERV <5), six mid-efficiency (MERV 5-8) and three high-efficiency (MERV 9-14) filters. A composite sample of living room and main bedroom floor dust was acquired from each building using a Dynamite Plus, Dirt Devil vacuum equipped with an Indoor Biotechnologies Duststream Collector. Approximately 1 m^2 of floor area was sampled for 2 minutes each, avoiding tracked-in dust areas. A composite high surface (horizontal surfaces > 1 m above the floor) sample was collected using the same vacuum technique from elevated surfaces such as door frames, shelves, and furniture. We collected three settled dust samples from sites 1, 2, 3, 4 and 7 and two samples from the remaining three sites. Two HVAC filters were collected from each site approximately three months apart, while the settled dust samples were collected

approximately four weeks apart from each other. In the same five buildings where three floor and high surface dust samples were collected, air samples were also collected from a height of 1 m to 1.5 m above floor level. An impinger (SKC Biosampler, Eighty Four, PA) was connected to a vacuum pump operating at a constant volumetric flow rate of 12.5 L min^{-1} for a period of 1 hour. The microorganisms were captured in a phosphate buffer solution (PBS) consisting of 8 g/L NaCl, 0.2 g/L KCl, 1.44 g/L Na_2HPO_4, and 0.24 g/L KH_2PO_4. All the samples were stored in a 4º C environmental chamber maintained at approximately 70% RH until the analyses were performed.

Sample Analysis

The enumeration of culturable microorganisms (both bacteria and fungi) present in the bioaerosol samples, settled dust, and HVAC dust samples were completed using the standard spread plate method 9215C (APHA, 1998). For the settled dust and the HVAC dust samples, the microorganisms present in the dust were transferred into PBS by sonication and vortexing for 10 minutes each. An aliquot of PBS was plated on R_2A agar containing 0.04 % cycloheximide and incubated at 30 °C for bacterial enumeration or on Sabouraud dextrose agar (SDA) plates containing 0.01% chloramphenicol and incubated at room temperature (approximately 23 °C) for fungal quantification. To estimate the spore-forming fraction of the population, an aliquot of each sample was pasteurized for 15 minutes at 75º C and then plated as described above. The Wilcoxon Rank-Sum Test, which does not assume any specific distribution of the data, was applied to compare and identify dissimilarities between the different data groups. A significance level of 0.1 was assumed owing to the small sample size and the conservative nature of this statistical test.

Phase 2: Microbial communities

In the second phase of the investigation, we expanded the analysis to a culture-independent, DNA-based approach potentially capable of more fully characterizing the microbial communities present indoors. This phase was conducted in a subset of four of the residential sites as well in an unoccupied 120 m^2 manufactured home (test house) where the fan of the HVAC system operated continuously during the investigation. The test house was mainly unoccupied, therefore reducing localized particle and microbial emissions and represented a good site to conduct detailed measurements. High-efficiency (MERV > 11) polyester HVAC filters were installed in all the sites at the beginning of this phase. At filter installation, several high surfaces were cleaned and the homeowners were instructed to not clean the delineated surfaces.

Sample Collection

Filters and high surface samples were collected two months after filter installation in the residences and one month after installation in the test house. At the time of filter removal, a composite sample of high surface dust from the previously cleaned surfaces was collected using the vacuum mechanism described above. During the month-long investigation in the test house, indoor and outdoor bioaerosol samples were also collected, 5 days per week, using the same impinger method described in Phase 1. Indoor bioaerosol samples were acquired from a central location in the building, while outdoor samples were collected at least 3 meters from any doors or windows. The DNA extracted from each daily bioaerosol sample in the test house was pooled together by combining equal volume aliquots of DNA extractions for the 20 daily samples into a composite integrated sample.

Sample Analysis

Approximately 50 mg of high surface dust sample was immersed into 50 ml of PBS, sonicated and vortexed for 10 minutes each to transfer the microorganisms to the liquid phase. Subsequently, the liquid solution was filtered first through a Whatman #41 (Whatman Inc., Piscataway, NJ) to remove large particles and then through a 0.2 µm GTTP Membrane Filter (Millipore, Billerica, MA) to separate the microbes from the particles, and the filters were stored at -80 °C until analysis. To sample the dust from each HVAC filter, nine 2.54 cm square pieces of filter material distributed in each quadrant were cut from the filter. Subsequently, they were all immersed into 50 ml of PBS, sonicated, vortexed and filtered as described above for the surface dust samples. For the bioaerosol samples, the PBS containing the microorganisms was directly filtered through a 0.2 µm filter.

The DNA from the microorganisms captured on the 0.2 µm filters was extracted using the Power Soil DNA (MoBio Laboratories, Carlsbad, CA) kit per manufacturer's specifications except for the following modifications. 100 µl of lysozyme (3 mg/ml) and 300 µl of a phenol-chloroform-isoamyalcohol (24:24:1) solution were added at the initial step in addition to the normal reagents. Also, the MP FastPrep-24 (QBiogene) was used instead of vortexing step. DNA samples were then PCR amplified using bacterial specific primers 8F (5'-AGAGTTTGATCCTTGGCTCAG-3') and 1492R (5'-GCYTACCTTGTTACGACTT-3') or fungal specific primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). These primers have been used successfully in several other microbial community studies (O'Brien *et al.*, 2005) and are useful for delineating and comparing the fungal community present in the samples collected from different indoor locations. Each 50 µl PCR reaction contained 1X PCR buffer, 1.6 mM $MgCl_2$, 0.2 mM each dNTP, 0.2 µM

each primer, 2 U of *Taq* polymerase, and 2 ul of DNA. The PCR amplification conditions consisted of 10 minutes at 94°C, followed by 35 cycles of 60 s at 94°C, 60 s at 55°C, and 60 s at 72°C and a final extension of 7 min at 72°C. For each sample investigated, we performed triplicate PCR reactions to reduce amplification biases; the amplicons were pooled prior to cloning. After confirming the amplicon length on agarose gel, the amplicons were purified using the QIAqiuck Gel Extraction kit (QIAGEN, Valencia, CA). Negative controls were performed and never showed amplification. Amplicons were then cloned using the TOPO TA cloning kit for sequencing (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions and subsequently sequenced in one direction with an ABI 3730 DNA analyzers (Applied Biosystems, Foster City, CA). The nonredundant sequences were deposited at the GenBank database with accession number GU595461-GU596375 for the bacterial clones and GU721174-GU722092 for the fungal clones.

Sequences were aligned against the GenBank database utilizing the BLAST algorithm (Altschul *et al.*, 1990) using 97% sequence similarity as the criterion to determine the similarity to known microorganisms. Sequences with lower similarity to a database match were classified as unknown but were still affiliated to the closest bacterial phylum or fungal subclass identified by the BLAST hits. The web-based tool FastGroupII (Yu *et al.*, 2006) was used to estimate the number of OTUs, Chao1 richness estimator and the Shannon-Wiener diversity index. The sequences from bacteria and fungi were then aligned separately using MEGA (Tamura *et al.*, 2007). The phylogenetic trees, containing the archeal sequence *Haloferax Volcanii* as an outgroup, were created in the CIPRESS portal (http://www.phylo.org/portal) using the RAxML algorithm (Stamatakis *et al.*, 2005). Finally, the microbial communities present in the different samples were compared using the Weighted UniFrac algorithm (Lozupone *et al.*, 2006;

Lozupone *et al.*, 2007). A significance level of 0.1 was assumed due to the limited number of sites and the exploratory nature of the investigation.

RESULTS AND DISCUSSION

Phase 1: Culturable microorganisms

Fig. 1 shows the mean bacterial and fungal culturable concentrations for each of the sampling locations investigated in the residences. Multiple samples at the same site are given equal weighting, so there are 21 samples each of floor and high surface dust, 16 HVAC filter dust samples and five air samples shown in the figure.

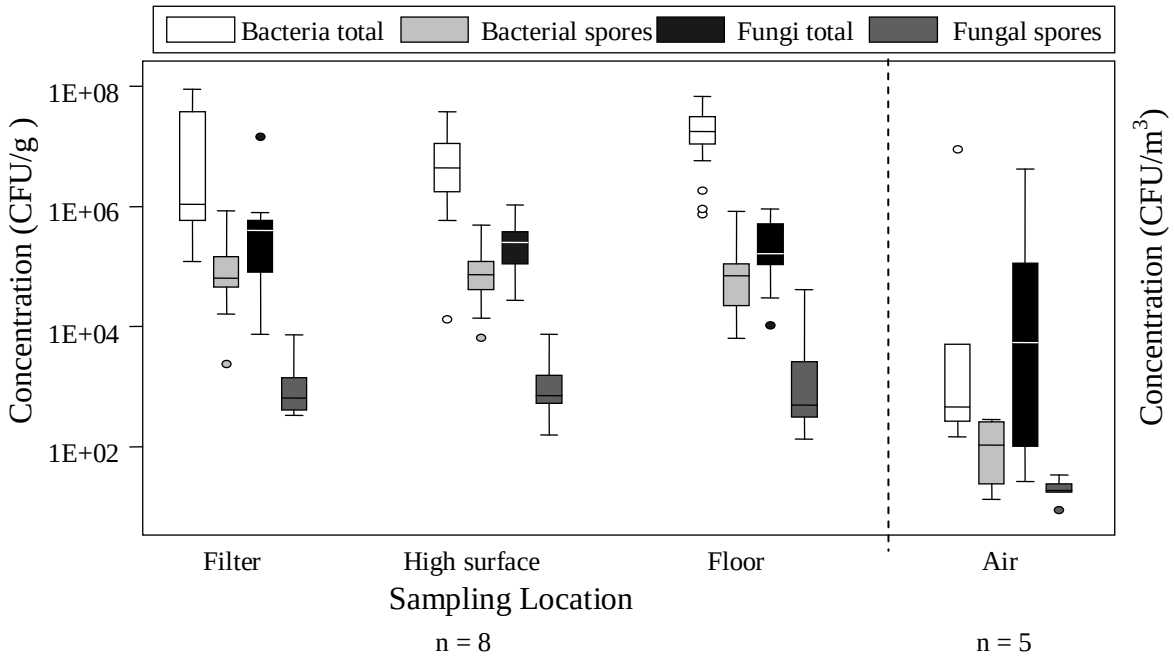

Fig. 1. Culturable microbial concentrations by sampling location. Air samples have dimensions of CFU/m^3 and all others have dimensions of CFU/g, with n= number of residences. The lowest end of the box represents the 25th percentile, the top represents the 75th percentile, and the horizontal bar inside the box indicates the median of the distributions. Single points outside the box are the outliers.

The culturable concentrations for both fungi and bacteria in Fig. 1 are generally consistent with the published literature. Indoor concentrations for bacteria and fungi vary

considerably with reported values ranging from 10^2 to 10^4 CFU m^{-3} for indoor air and from 10^5 to 10^7 CFU g^{-1} for settled dust (Andersson *et al.*, 1999; Bouillard *et al.*, 2005; Dales, 1997; Koch *et al.*, 2000; Ren *et al.*, 1999; Ross *et al.*, 2000). For all of the sampling locations, viable bacterial concentrations are higher than fungal concentrations and the estimated spore concentrations are approximately two orders of magnitude lower than total concentrations. Total bacteria concentrations range from 10^4 to 10^7 CFU/g, with a greater median concentration found on the floor, followed by high surfaces, and HVAC filter samples with median concentrations of 1.9×10^7, 4.4×10^6 and 1.1×10^6 CFU/g, respectively. This would suggest that larger particles or clusters of bacterial cells that are more likely to settle may have greater bacterial concentrations than small particles that remain suspended in air and are captured on the filter. Another possible explanation could be that the survival/growth conditions and nutrient availability on surfaces may be more favorable than on the filters. Fungal concentrations in the dust samples ranged from 10^3 to 10^7 CFU g^{-1} with reasonably consistent distributions across the dust sampling locations. It is important to note that airborne microorganisms may be attached to particles, and the size of the particles to which they are attached may have the greatest influence on their fate in an indoor environment (Hairston *et al.*, 1997).

A greater variation in fungal spore concentrations was observed in the floor dust samples, possibly due to the different types of flooring (i.e., carpet and hardwood floor) present in the different residences. However, there was a small variation in the bacterial concentrations suggesting that other factors beside floor surface characteristics may be important. In the air samples, there the median fungal concentration was greater than the median bacterial concentration and the concentration of culturable fungi varied considerably.

Several studies showed that indoor air fungal concentrations have elevated temporal and spatial variability (Hyvärinen *et al.*, 2001; Koch *et al.*, 2000) and thus the short sampling time may have affected the results. Stanley *et al.* (2008) calculated low indoor air culturable concentrations for selected bacterial species, often below 4 CFU/m^3, based on HVAC filter concentrations. The results in the current study may diverge from those due to differences in quantification techniques and the fact that HVAC systems in the current study supplied 100% recirculated indoor air and operated intermittently when the thermostat called for conditioning.

Fig. 2 summarizes the mean culturable concentrations of bacteria and fungi in the floor dust, high surface dust and HVAC dust at each of the eight sites investigated. The concentration of bacteria was fairly consistent within one order of magnitude across most sites except for Sites 1, 2 and 3. At Site 3, HVAC filter and high surface dust concentrations were quite similar but the floor dust samples had much greater concentrations and may have been influenced by tracked-in particles from outside. The difference between the HVAC filter and the high surface dust samples at Sites 1 and 2 may be due to the reduced efficiency of the filters collected from these sites, specifically one low- and one mid-efficiency filter for Site 1 and two low-efficiency filters for Site 2. The reduced efficiency of these filters makes them less ideal sampling devices and increases the probability of observing differing levels on the filters than on the floor or high surface. The difference in microbial concentrations on the filters and those found in surface and floor dust at these three sites may also be attributable to the cycling of the HVAC system (Noris *et al.*, 2009), suggesting that HVAC filters in residential buildings where the HVAC system is operated sporadically may be less representative of indoor contaminant levels. Nevertheless, despite some site-specific differences, the Wilcoxon

sign-rank test reveals that the culturable microbial concentrations encountered at different dust sampling locations for all the sites were not statistically different.

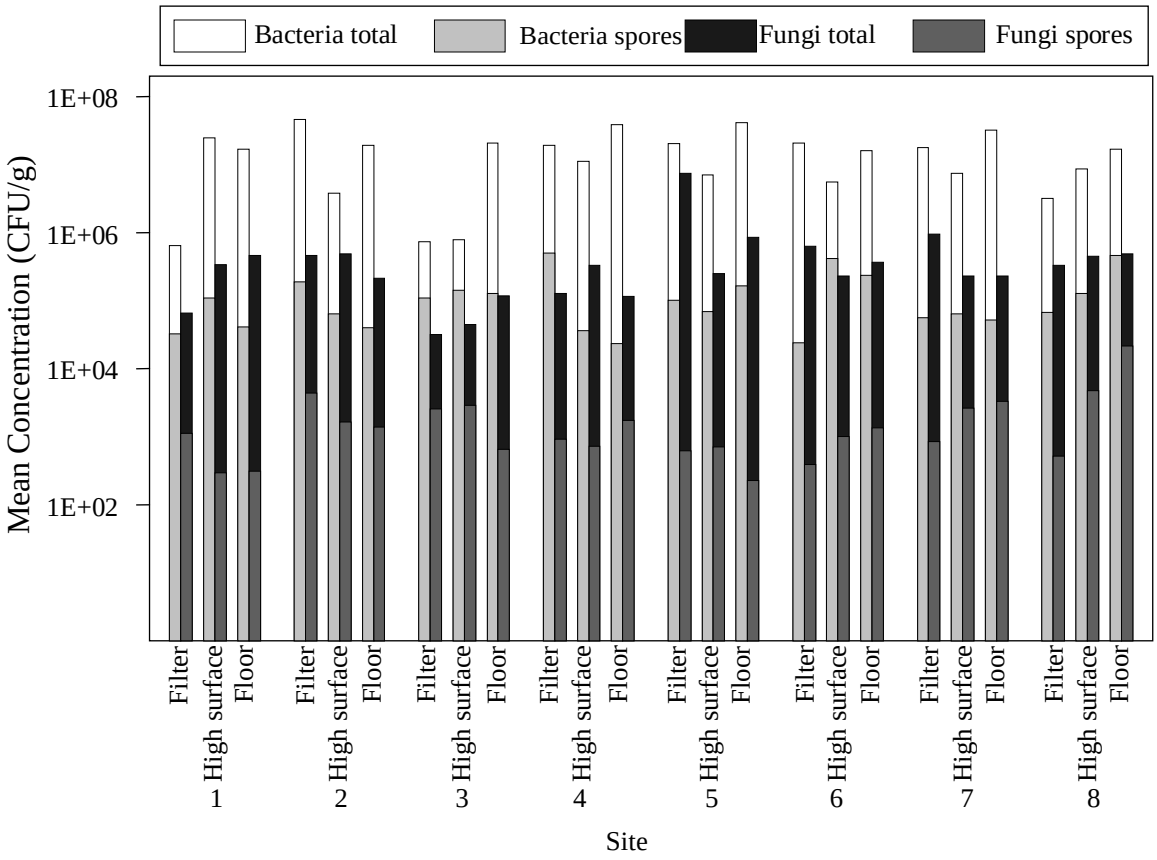

Figure 2. Mean culturable microbial concentrations in dust samples by location within building.

From Fig. 1 and 2 it is clear that both bacteria and fungi are able to populate and survive in the dust present indoors. Importantly for this work, in a humid and warm environment like central Texas during the cooling season, microorganisms appear to survive and colonize the dust on HVAC filters with concentrations similar to those found in the dust that settles inside the residences, suggesting that these filters may be a

promising location for collecting samples for indoor assessments. While the culturable concentrations were comparable, the compositions of the microbial communities may differ with sampling location because of specific environmental conditions that may favor some species over others. This aspect, as well as the influence of occupants on the composition of indoor microbial communities, is addressed in Phase 2.

Phase 2: Microbial communities

Following the investigation of bacterial and fungal culturable concentrations in residential sites, we then expanded the study to the microbial community analysis on a subset of four residential sites and in a mostly unoccupied full-scale test house using a DNA-based approach. Additionally, the identification of microbial species common on HVAC filters as well as the association between occupants and microbial species could also be performed. After eliminating all potentially chimeric, or poor quality sequences, we obtained a total of 915 bacterial clones and 919 fungal clones, corresponding to 248 and 295 Operational Taxonomic Units (OTUs), respectively. The bacterial clones had an overall Chao1 value of 426 and a Shannon-Wiener index of 4.58, while fungal clones had values of 508 and 4.62, respectively. These values indicate a microbial representation similar to what observed in other indoor studies (Pitkäranta *et al.* 2008; Rintala *et al.*, 2008; Täubel *et al.*, 2009).

Fig. 3 summarizes the bacterial composition at the phylum level for all the samples analyzed. The most common phyla encountered are gram-negative Proteobacteria, and gram-positive Actinobacteria and Firmicutes, a finding which is in agreement with recent DNA-based studies by Rintala *et al.* (2008) and Täubel *et al* (2009). These three phyla represent 96% of the clones encountered on the residential filters and 90% of the clones found in the high surface residential samples in the current

study. For all the residential sites investigated, Proteobacteria were present in greater proportion in the filter dust samples than in the high surface samples, with mean values of 65% and 39% respectively. Tringe *et al.* (2008) utilized a DNA-based technique similar to the current study and also observed an elevated proportion of Proteobacteria on HVAC filters in two commercial buildings. These results contrast to those reported by Stanley *et al.* (2008) who observed that the gram positive *Bacillus* (of the Firmicutes phylum) was the most commonly identified group in a culture-based study of HVAC filter bacterial communities. Thus, the prevalence of gram-positive bacteria in the Stanley *et al.* (2008) study may be due to a bias of culturing techniques that favor gram-positive bacteria. The results from culture-independent studies described herein and by others suggest that Proteobacteria represent a significant fraction of the indoor air bacterial community and that this phylum may better tolerate the environmental conditions encountered in air (Brodie *et al.*, 2006; Fierer *et al.*, 2008) and on HVAC filters. One explanation could be that they possess a greater fraction of key genes involved with resistance to desiccation and oxidative damage, as suggested by Tringe *et al.*, (2008). While Proteobacteria dominated the filter dust samples, an opposite trend was observed for Actinobacteria, with the mean percentage in the high surface samples more than four times higher than that found on the filters, 26% versus 6%.

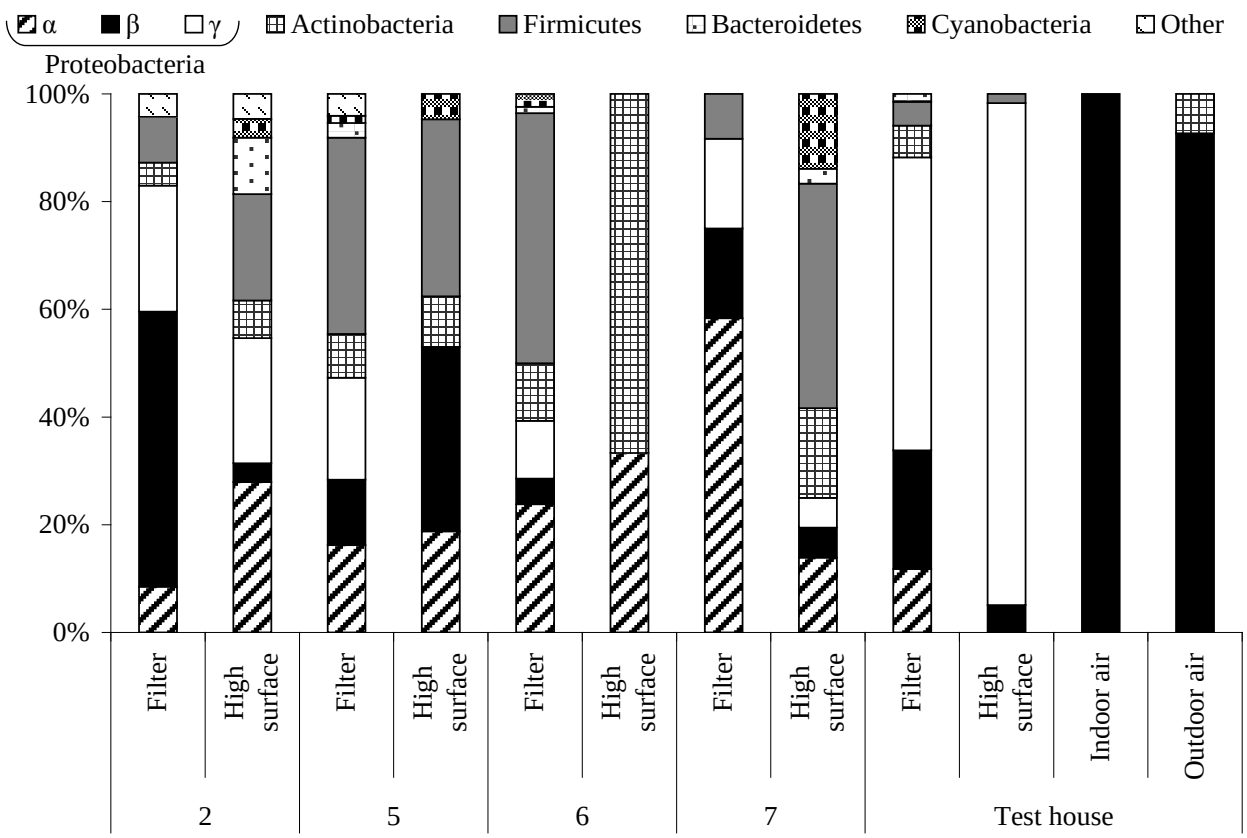

Figure 3. Bacterial composition at the phylum level for the sequence libraries obtained from all the samples analyzed.

Comparison of the clone libraries generated from the dust samples in occupied residences to those in the unoccupied test house indicates that a much greater proportion of gram-positive bacteria, mainly Firmicutes and Actinobacteria, were present in the residences versus in the test house, with mean values of 41% and 6% respectively. This increased proportion of gram-positive bacteria in occupied buildings supports the speculation that many gram-positive bacteria found indoors may be attributable to human sources (Horak *et al.*, 1996; Pakarinen *et al.*, 2008; Rintala *et al.*, 2008; Täubel *et al.*, 2009). Rintala *et al.* (2008) examined the bacterial communities in surface dust in two buildings across seasons. They observed higher variation in microbial composition between buildings than between seasons suggesting the development of site-specific

bacterial communities, and that building users may be responsible for the presence of the dominant bacterial groups. A similar suggestion was made by Täubel *et al.* (2009) after examining the bacterial communities in mattress dust, floor dust and skin surface samples of occupants in four residences. Also, there was a greater proportion of gram-negative bacteria, primarily Proteobacteria, in the dust samples collected in the test house (mean value of 93%) versus those collected in the residences (52%), corroborating the supposition that gram-negative bacteria, and specifically Proteobacteria, may be of outdoor (i.e., environmental) origin. In the test house we observed a dominance of Proteobacteria for all the samples analyzed. Fierer *et al.* (2008) reported elevated temporal variability in outdoor samples with a dominance, across the five sampling days, of Proteobacteria and Bacteroidetes. This latter phylum was rarely observed in the current study and may be more typical of colder climates (Miteva *et al.*, 2004; Yi *et al.*, 2005). An elevated presence of Proteobacteria in outdoor air communities was also reported by Brodie *et al.* (2006) for the same geographic area of this study, confirming that this may be the most abundant phylum in ambient air samples.

Fig. 4 shows the fungal composition at the subclass level for all the clone libraries acquired. The majority of the sequences belonged to the phylum Ascomycota, with a much smaller fraction assigned to the Basidiomycota phylum. The majority of the fungal clones encountered in the samples analyzed belong to the *Dothideomycetes* (*Pleosporomycetidae*, *Dothideomycetidae* subclasses), *Sordariomycetes* (*Hypocreomycetidae*, *Sordariomycetes incertae sedis*) or *Agaricomycetes* (*Agaricomycetes incertae sedis*) class. Specifically, the *Dothideomycetes* class seems to be dominant, with *Cladosporium* and *Alternaria* spp. being the most abundant representatives. Pitkäranta *et al.* (2008) also observed an abundance of the *Dothideomycetes* class in indoor dust from two nursing homes in Finland even though the

most common phylum was Basidiomycota. However, they also observed an increase in Dothideomycetes, and therefore in Ascomycota, during the summer months which represent a more similar climate to that encountered in central Texas in summer and fall. In the *Sordariomycetes* class, the genus *Fusarium* spp. was the most commonly detected which is consistent with results of other studies (O'Brien *et al.*, 2005; Pitkäranta *et al.*, 2008) which also used a molecular-based approach. Some culture-based studies reported elevated concentrations of the genera *Penicillium* and *Aspergillus* spp. in indoor and outdoor communities (Koch *et al.*, 2000; Ren *et al.*, 1999). However, in the current study, we observed a limited proportion of the class corresponding to these genera, *Eurotiomycetes*. This discrepancy could be due to a specific bias of the culturing methods that favor these species.

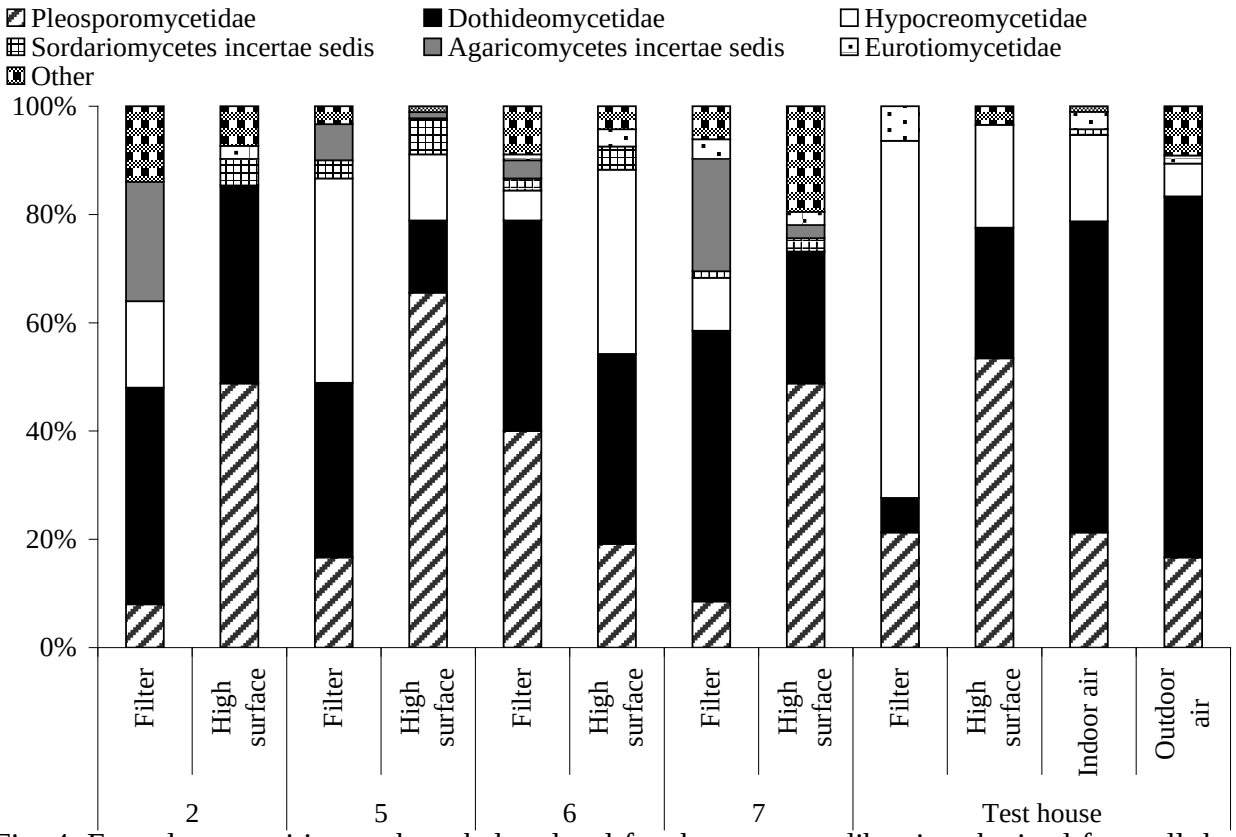

Fig. 4. Fungal composition at the subclass level for the sequence libraries obtained from all the samples analyzed.

When comparing the fungal composition within a given site, we observed that, for all the sites except Site 6, the proportion of *Dothideomycetes* is greater in high surface dust (a mean of 76%) than in filter dust samples (a mean of 59%). An opposite behavior is observed for *Agaricomycetes* that are present in filter dust samples in much greater proportion than in high surface samples for all the residential sites, with mean values of 16% and 1%, respectively. *Sordariomycetes* are present in a greater proportion in filters than in the high surface dust samples. This is especially true for the test house filter where this class seems to proliferate constituting 66% of the fungal clones obtained. The proportion of *Sordariomycetes* increased from a mean value of 19% to 42% between all the dust samples in the residences and those in the test house. This class has been

observed to dominate in outdoor air samples (Fierer *et al.*, 2008) confirming the potential environmental origin of this class in the test house. However, the fraction of this class was not particularly high in indoor air, but seems to proliferate in the test house filter, therefore other factors may be important. Both indoor and outdoor air samples were dominated by ascomycetes, as also reported by Fierer *et al.* (2008) for outdoor air, supporting the hypothesis that indoor fungal communities strongly depend on outdoor fungi microbiota (Pitkäranta *et al.*, 2008).

Some of the clones from the occupied residences have high similarity to species that are reported to be potential opportunistic pathogens. These species include for Bacteria *Pantoea agglomerans*, *Ralstonia pickettii*, *Enterobacter hormaechei*, *Staphylococcus aureus* and *epidermidis*, as well as *Bacillus cereus*, *pumilus*, and *subtilus*. Fungal potential pathogens include *Alternaria alternate* and *tenuissima*, *Fusarium proliferatum* and *oxysporum*, *Nigrospora shaerica*, and *Cladosporium cladosporioide.*, The presence of these opportunistic pathogens on HVAC filters confirm the potential application of filters as samplers for detecting harmful microorganisms. However, additional analyses will be required to determine if these microbes were actually in a viable state.

In order to evaluate the potential use of HVAC filters as a sampling mechanism for indoor microbial communities, the similarity between microbial communities in different indoor sampling locations was evaluated using the UniFrac significance metric. Table 1 presents the UniFrac values for the comparisons between the HVAC filter and high surface dust samples in the residential sites. From the p-values, it appears that, although some differences in composition are present (Figs. 3 and 4), both bacterial and fungal communities in the filter and high surface dust samples within each residence investigated are not statistically different. Thus, the UniFrac results suggest that in a

given residence, the microbial community present in high surface dust is similar to that present in HVAC filter dust and high-efficiency filters may be suitable samplers for assessing the composition of indoor microbial communities. This similarity seems to contradict some of the compositional findings described above, possibly because of the nature of the Unifrac analysis, which relies on phylogenetic information rather than a species by species comparison.

While the UniFrac comparisons indicate that the microbial communities in HVAC filter dust and high surface dust are similar in a given residence, Site 7 had the lowest p-values of all the residences for both the bacterial and fungal community comparison, with a bacteria p-value of 0.10, the threshold of significance. One possible explanation is the location of the HVAC filter in this residence. The filter was located at the return grille in the hallway, away from some of the rooms where the high surface dust sample was collected (living room and two of the four bedrooms) potentially resulting in differences in the particles collected on the filter versus those being deposited on indoor surfaces. The fungal and bacterial concentrations determined using culturing techniques (Fig. 2) are similar for the filter and high surface dusts at Site 7, while greater variations in the microbial concentrations between filter and high surface dusts are observed at other sites (i.e., bacteria for Site 2, fungi for Site 5) that had similar microbial communities (Table 1). This lack of correlation between culture-based and culture-independent results highlights the fact that similarity in culturable concentrations is not necessarily associated with similarities in the microbial communities. When we monitored the HVAC system usage for 2-3 day-long monitoring periods during Phase 1 (Noris *et al.*, 2009), Site 7 had the highest cooling duty cycle (34% compared to a median of 18% for the other sites), suggesting that other factors other than the HVAC system usage could be important. More intuitively, for the other two sites where a high cooling duty cycle was found, Site 5

(32%) and 6 (29%), the p-values are well above the threshold. However, the authors suggest caution in interpreting these results because of lack of direct monitoring of HVAC operation during the measurements described here.

Table 1 UniFrac significance value for the filter to high surface comparison in the four residential sites.

	UniFrac Significance (P-value)			
	Site number			
	2	5	6	7
Bacteria	0.41	0.35	0.48	0.10
Fungi	0.30	0.65	0.55	0.16

Table 2 shows the UniFrac significance values for the samples collected in the unoccupied full-scale test house. The p-values for the microbial community comparisons between the filter and high surface dust samples (second column) are lower than those determined for the residences (Table 1) suggesting that human-associated microorganisms dominate the communities in occupied residences, as reported by others (Rintala *et al.*, 2008; Täubel *et al.*, 2009). This could be due to the fact that in residences, occupants generate particles through their activities and introduce microorganisms that could deposit onto surfaces or be captured by the filter leading to a more homogeneous distribution of microorganisms in the indoor environment. In contrast, the fungal communities in the HVAC filter and high surface dust samples in the test house are statistically different, while bacteria are not, suggesting the fungi may be more prone than bacteria to develop communities adapted to the specific environment. The difference in the fungal communities observed in filter and high surface dust in the test house could be attributable to the increased proportion of Sordariomycetes in filter dust.

Table 2 UniFrac significance values for the samples collected in the test house

UniFrac Significance (P-value)

	Filter vs. high surface	Filter vs. indoor air	High surface vs. indoor air	Indoor vs. outdoor air
Bacteria	0.13	0.14	**0.01**	0.12
Fungi	**0.01**	0.24	**0.03**	0.19

The results from the month-long investigation in the unoccupied test house indicates that the filter and the composite indoor air sample (third column) were not statistically different supporting the findings of Tringe *et al.* (2008) that suggested that HVAC filter dust can be used as an integrated measure of airborne microbial communities, even though some specific differences in the community may occur. HVAC filters are in place for extended periods of time, therefore, during their usage, a great volume of air is filtered through them (Stanley *et al.*, 2008) and only the microorganisms that were, at some point in time, airborne have the opportunity to be captured on the filter. In this study, the airborne microbial communities derived from the analysis of a composite sample of 20 daily 1-hour samples represent a more integrated measurement. Short-term air samples are reported to have a great temporal variability (Brodie *et al.*, 2006; Fierer *et al.*, 2008) and by compositing daily collections we tried to overcome this limitation so as to be able to compare the indoor air samples to the dust that collected on the surfaces and on the filter over the same period.

The values reported in the fourth column of Table 2 indicate that both bacterial and fungal communities in high surface dust and indoor air were statistically different in the unoccupied test house. Therefore, high surface dust samples are not representative of airborne microbial communities possibly because surface dust samples may be influenced by the microorganisms attached to the particles that are more likely to deposit on surfaces (i.e., larger particles) rather than stay in air. Single bacterial and fungal cells range from 0.5 μm to 50 μm, with fungal spores generally larger than bacterial spores (Li and Li, 1996; Terzieva *et al.*, 1996). The size of these biological particles influences their fate

and the probability of being detected in the different sampling locations, since larger particles are likely to settle while smaller particles may stay longer in air and have more opportunities to be captured on HVAC filters. In the residences, the presence of occupants and the microorganisms associated with them tend to homogenize the communities. In the test house, these phenomena are more evident due to the limited occupancy. The significantly different bacterial community between these high surface and indoor in the test house seems to be largely attributable to the different composition of Proteobacteria. Air samples were dominated by β-Proteobacteria with 100% and 93% of the clone libraries for, respectively, indoor and outdoor air samples. In contrast, test house dust samples seem to be dominated by γ-Proteobacteria which constituted 54% of the bacterial clones encountered on the filter dust and 93% of those found on high surface dust sample (Fig. 3). For fungi the difference between high surface and air may reside in the composition of the *Dothideomycetes* class. The high surface sample is constituted by 53% of the subclass *Pleosporomycetidae* and 24% of the subclass *Dothideomycetidae*, while for indoor air the proportion for these two subclasses is inverted, with the former accounting for 21% and the latter 57% of the sequences (Fig. 4). Finally, we observed similar microbial communities in indoor and outdoor air (last column of Table 2). Most importantly for this investigation, the findings reported in Table 2 confirm that high-efficiency HVAC filters located in HVAC systems operating a great fraction of time can be used as a surrogate for long-term air samples that could be use as an alternative to extensive periodic air sample collections with not statistically different information. Given the results from the four occupied sites, we would anticipate that these results would also hold for occupied environments.

CONCLUSIONS

This study evaluated the use of HVAC filters as long-term air samplers for indoor biological contamination. Microbial concentrations and communities on HVAC filter dust samples were not statistically different from high surface dust samples in residences. However, differences in the community compositions may exist between samples collected in different indoor locations and between occupied and unoccupied buildings. Proteobacteria were present in greater proportion on HVAC filter dust samples than in high surface dust samples and in the unoccupied test house than in residences suggesting the outdoor air origin of this phylum. Gram-positive bacteria were present in greater proportion in occupied residences than in the unoccupied test house, confirming the potential association of this group with occupants. HVAC filter microbial communities were not statistically different from a composite indoor air sample in a mostly unoccupied test house. The results indicate that HVAC filters may be a viable option for investigating indoor biological contaminants and could be used as surrogate for long-term air samples, as suggested by other researchers. The current study represents an exploratory investigation of the potential use of HVAC filter as sampling mechanism for indoor microbial communities. The results are promising and suggest that a more comprehensive investigation of this technique is warranted.

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Appendix C

PAPER III

FATE ANALYSIS OF INDOOR PARTICLES AND EVALUATION OF HVAC FILTERS AS SAMPLERS

(In preparation for submission to Building and Environment)

ABSTRACT

HVAC filters are in place for extended periods of time and could serve as long-term samplers. To assess the potential use of HVAC filters as passive indoor samplers in a typical residence, we conducted a scaling analysis to evaluate the removal of particles resulting from deposition, exfiltration, and capture in the HVAC filter. Experiments under controlled conditions were conducted in a full-scale test house to confirm the validity of the model. In the model, typical characteristic times for each removal mechanisms were applied to indoor airborne particles in the 0.001-100 μm range to assess the effectiveness of using filters as samplers. The results suggest that large particles are likely to deposit, particles in the 0.03-3 μm range are likely to be removed by exfiltration or, if a high efficiency filter is installed, to be captured by the HVAC filter. Ultrafine particles (< 0.1 μm) are also likely to be captured by filters, particularly for elevated recirculation air exchange rates. HVAC filter efficiency and the recirculation air exchange rate play a key role in the use of HVAC filters as representative samplers of indoor particles. High efficiency filters with elevated recirculation air exchange rates (>5.2 h^{-1}) are particularly effective for a wide range of particle sizes suggesting that HVAC filters may be a promising means for assessing indoor particulate contaminants.

KEYWORDS

Particulate matter, Filtration, Air sampling, Settled dust, Removal mechanisms.

INTRODUCTION

Indoor air quality investigations often focus on air and settled dust samples to assess chemical and biological contamination. Although the information provided by these techniques is useful, both types of sampling locations have limitations including the spatial variability of indoor contaminant concentrations. Additionally, air samples are short-term in nature providing only a snapshot of contaminant concentrations. The specific contaminants and concentrations found in particles suspended in air and in settled dust are often different (i.e., Rudel *et al.,* 2003) possibly due to the fact that air and settled dust sampling methods preferentially sample different particle size ranges. Settled dust may be biased toward larger particles that are likely to deposit by gravity onto surfaces. Additionally, floor dust may be influenced by tracked-in dust of outdoor origin. In contrast, air samples may tend to preferentially collect particles with sizes that are not effectively removed by other mechanisms such as deposition and filtration.

In order to compare the contaminant concentrations observed in samples collected from various indoor locations, the fate of indoor airborne particles and their likelihood to be removed by different mechanisms needs to be investigated. The fate of indoor airborne particles is a complex phenomenon with several competing mechanisms that are influenced by a variety of parameters, including the specific characteristics of the building and of the HVAC filtration system as well as by the particle size of interest. The main indoor particle removal mechanisms are deposition onto surfaces, exfiltration through the building envelope and, if the HVAC system is being operated, HVAC filtration. Particle deposition onto indoor surfaces as a function of particle size has been widely studied (Long *et al.* 2001; Riley *et al.*, 2002; Thatcher and Layton, 1995) mostly in chamber studies and in controlled environments. Riley *et al.* (2002) reported that loss processes vary with building conditions and operation and are strongly particle-size

dependent. Air exchange rate and exfiltration of particles affect indoor particle concentrations as reported by Abt *et al.* (2000). HVAC filters are capable of removing indoor airborne particles (Hanley *et al.*, 1994) and play a critical role in the decay of particle concentration in indoor environments (Fisk *et al.*, 2000). Wallace *et al.* (2004) investigated the impact of a central fan and mechanical filters and reported that filters can effectively reduce indoor air concentrations with increased particle removal rates by up to 2 h^{-1} for fine and ultrafine particles. Siegel and Waring (2008) observed the influence of HVAC filter efficiency, time of operation and particle size on the loading rates of HVAC filters. Zhao and Wu (2009) investigated particle fate in ventilation systems, including filters, for a range of different scenarios reporting a strong dependency on particle size. To evaluate the merits of utilizing HVAC filters as passive samplers, the current study expands on this particle fate analysis to assess the likelihood of particle capture on filters for a range of building and HVAC scenarios.

HVAC filter dust has received little attention as a potential resource for indoor environment investigations. These filters are in place for extended periods of times and throughout their life can collect an integrated sample of particles present in the indoor environment. Analysis of HVAC filter dust may enhance our understanding of indoor occupant exposure by providing an integrated measure of indoor pollutant concentrations associated with particles. Recently, several researchers have utilized HVAC filters as a sampling mechanism for airborne particle-bound contaminants such as microorganisms and metals (Noris *et al.*, 2009; Stanley *et al.*, 2008; Tringe *et al.*, 2008). The objective of the current study was to validate a modeling approach capable of predicting the removal probabilities of indoor particles by the different mechanisms. The model was then applied to a typical residential scenario and the influence of filter efficiency, air recirculation rate, and air exchange rate on the size-dependent particle fate was evaluated.

The likelihood of HVAC filters to collect particles was evaluated in order to delineate the conditions and particle sizes for which HVAC filters are most likely to be effective samplers. Additionally, we wanted to compare the use of HVAC filters to more traditional sampling approaches such as periodic air measurements or of settled dust collection. The results from this analysis will be useful for assessing the effectiveness of using HVAC filters as an indoor sampling technique.

MODEL DESCRIPTION AND PARAMETERS

A scaling analysis was performed to estimate the probability that 0.001-100 μm particles would be removed from indoor air in a typical residence by deposition, exfiltration, or filtration through the HVAC filter. The volume of the residence was selected considering a typical floor area of 163.3 m^2 (US Bureau of Census, 2005) and assuming a ceiling height of 2.4 m for a total volume (V_T) of 391.9 m^3. To estimate the removal probability for each mechanism, we considered the size-dependent characteristic time for each removal process. The characteristic time for deposition was the particle size-resolved deposition loss rate coefficients (β), for exfiltration, the air exchange rate (λ) was utilized, while for filtration, the recirculation rate (λ_r) multiplied by the size-dependent removal efficiency (η) of the HVAC filter was used.

Model parameters were estimated from the literature. For deposition, the β values summarized by Riley *et al.* (2002) were utilized. For exfiltration, we utilized the 10^{th}, 50^{th} and 90^{th} percentile values of the λ distribution reported by Murray and Burmaster (1995). These values, corresponding to λ = 0.2, 0.5 and 1.3 h^{-1}, were used to evaluate how the tightness of the residence may affect the potential use of HVAC filters as passive samplers. For the loss rate due to filtration, the λ_r multiplied by the size-dependent removal efficiency (η) of the HVAC filter was utilized. We considered two different λ_r

values (5.2, 1.1 h^{-1}) by assuming either continuous operation for mechanical ventilation (λ_r= 5.2 h^{-1}) or cyclic duty operation (λ_r= 1.1 h^{-1}) for a typical 3-ton air conditioner operating 22% of the time (Noris *et al.*, 2009). Three different clean filters with minimum efficiency reporting values (MERV), as determined by ASHRAE Standard 52.2 (ASHRAE, 2007), of <5, 6 and 11 were considered using the filtration efficiencies employed by Waring and Siegel (2008). For each scenario, *j*, considered, the size-dependent characteristic times for each mechanism were then normalized by the sum of all the characteristic times, k_j, and the resulting fraction represented the relative removal probability of that mechanism for a given particle size. . The sum of the characteristic times, k_j, was calculated as follows:

$$k_j = \beta + \lambda_j + \lambda_{r,j}\eta_j \quad (1)$$

As a consequence, for a particular scenario, the size-dependent particle removal probability via each mechanism, $p_{r,m}$, was estimated with the model as follows:

$$p_{r,m} = \frac{r_{j,m}}{k_j} \quad (2)$$

where $r_{j,m}$ is the size-dependent characteristic time of each process for a particular scenario. Particle deposition in the HVAC system ducts and coil was neglected based on the results of previous studies (Sippola and Nazaroff, 2003; Waring and Siegel, 2008). We assumed isothermal conditions and ignored particle resuspension, coagulation and phase change.

METHODOLOGY

The validation of the modeling approach was performed in a 110 m^2 (volume of 250 m^3) unoccupied test house. The house was equipped with a 2.5-ton (8.8 kW) air

conditioning system that was continuously operated during the experiments. The system airflow was measured using a TrueFlow metering plate and DG-700 digital manometer (Energy Conservatory, Minneapolis, MN) connected to a pressure tap in the supply plenum. Approximately 50 g of Ultrafine Arizona Test dust (Powder Technology, Burnsville, MN) were dispersed into the house using a dust sprayer and mixing fans. Eight mixing fans and one ceiling fan were operated to improve the mixing of the injected particles. Six Aerotrak Handheld Particle Counters (TSI Inc., Shoreview, MN) were located in different indoor locations: living room, kitchen, upstream and downstream of the HVAC filter and one in each of two bedrooms. We measured particle concentrations in the following size bins: 0.3-0.5, 0.5-1, 1-3, 3-5, 5-7, 7-10 μm. Samples were recorded at 30-second intervals. Experiments with high (MERV 12) and low (MERV 2) efficiency fibrous filters were conducted in triplicate. Each experiment lasted approximately 120 min. To estimate the mass accumulated on each filter, we weighed the filters before and after the experiment using a balance (Sartorius B310S, Goettingen, Germany). Prior to the beginning of the experiments, particle decay tests were performed to estimate the removal of particles due to deposition onto surfaces when the HVAC system was off. For these particle decay tests, approximately 10 g of dust was sprayed and the concentrations were measured as explained below. In this way, the deposition loss coefficient (β) in the test house could be determined for conditions similar to those present during the tests.

At the beginning of each experiment, all surfaces and floors were cleaned and a clean filter was installed. Approximately 4 m^2 of selected high surfaces (horizontal surfaces > 1 m above the floor) and 6 m^2 of floor surface were sampled using a Dynamite Plus vacuum (Dirt Devil, Glenwillow, Ohio) equipped with a Duststream Collector (Indoor Biotechnologies, Charlottesville, VA). The particle mass deposited on high

surfaces and on the floor was measured by weighing the collectors before and after the sampling. During the tests, the house was pressurized using an Duct Blaster (Energy Conservatory, Minneapolis, MN) equipped with two MERV 11 filters and an activated carbon mat to remove outdoor particles and particle forming compounds. During each test, the air exchange rate was assessed by the best fit to exponential decay of CO_2 concentrations verses time, correcting for background CO_2. The CO_2 concentrations were monitored in several locations inside and outside the house using Telaire 7001 Carbon Dioxide Monitors (GE, Billerica, MA). The size-resolved number of particles removed by filtration, n_f, was estimated using the following equation for filtration:

$$n_f = \sum_{t=0}^{t=120} \left(C_{up} - C_{down}\right) Q_r t \qquad (3)$$

where C_{up} and C_{down} are the measured size-resolved concentrations (#/m^3) upstream and downstream of the HVAC filter, Q_r is the flow rate (m^3/h) through the filter, and t is the duration of each experiment (h). The size-resolved n_f was then multiplied by a characteristic volume for each size bin (assuming spherical particles) to obtain the volume of particles collected on the filter, v_f. Using the particle size distribution provided by the manufacturer for the Ultrafine Arizona Test dust, the size-resolved volume of particles injected, v_i, was calculated assuming a constant density across particle size ranges. The ratio between v_f and v_i represents the estimated probability of filter removal for each size bin during the experiments, $p_{f,e}$:

$$p_{f,e} = \frac{v_f}{v_i} \qquad (4)$$

RESULTS AND DISCUSSION

Results from validation experiments

The air exchange rates during the experiments ranged from 1.57 to 1.96 h^{-1}, while the HVAC flow rate (Q_r) was approximately 1530 m^3/h and 1630 m^3/h for the high and low efficiency filters, respectively. The majority of the injected particles were removed from the air in the first 10-15 minutes. With the exception of the submicron particles, 90% of the particles in the other four size bins, that were ultimately collected on the filter, deposited on the filter within the first 20 minutes after particle injection. Submicron particles tend to be removed more slowly and, as a consequence, HVAC filters may have limitations for an application that requires rapid detection of contaminants in this particle size range. Table 1 shows the comparison between the fraction of the injected dust mass that was collected on the filters and the particle volume fraction calculated using Equation 4. Since we assumed a constant density across particle size ranges, the mass fraction and the volume fraction represent the same metric and can be compared. The volume percentage estimated using Equation 4 matches the measured mass fraction on the filters within 10%, except for the Test 3 high efficiency filter and the Test 3 low efficiency filter. During these two experiments, the equation overestimated the fraction of the injected particles captured on the filter, possibly because of nonuniform mixing conditions throughout the house. For these two experiments, the standard deviations of the indoor concentrations measured in the house normalized by the initial concentrations were the highest values of all the experiments. This would lead to a greater variation (positively or negatively) between what is captured on the filter and what is predicted by the model which assumes a well-mixed condition. The particle mass collected on the floor and high surfaces varied more between tests than did the mass collected on the filters. During the experiments with high efficiency filters, we calculated that between 57% and 76% of the total mass of particles injected in each test deposited on surfaces, while for the low efficiency experiments this percentage was between 67% and 83%.

Table 3. Comparison between measured mass and calculated volume fraction of injected particles on HVAC filters

Test	Measured Mass Fraction (%)	Calculated Volume Fraction (%)[1]
High efficiency filter test 1	20.0	20.9
High efficiency filter test 2	19.2	19.9
High efficiency filter test 3	14.4	18.5
Low efficiency filter test 1	8.47	8.56
Low efficiency filter test 2	9.15	10.8
Low efficiency filter test 3	7.96	10.9

[1]This fraction is calculated using the upstream and downstream filter concentrations and Equations 3 and 4.

Figure 1 presents the comparison between the filter capture probabilities determined during the experiments (and calculated using Equation 4) and the model prediction for the low and high efficiency filters. The model utilized the actual λ, Q_r and V_T values measured during the experiments; it also employed the deposition loss rate (β) and the filter efficiency (η) values obtained from the literature (Riley *et al.*, 2002; Waring and Siegel, 2008) While the λ, Q_r and V_T are parameters relatively easy to obtain and measure, measuring β and η requires particle injection tests which are time consuming and more complex to perform. Thus, we were interesting in evaluating the applicability of the model if the β and η parameters are not measured directly but rather estimated from literature values. The model used the mean of the λ, Q_r measured during the three tests. These values corresponded for the high efficiency filter model predictions to λ= 1.74 $h^{-1}$and Q_r= 1529 m^3/h,, while for low efficiency filter model predictions we utilized λ= 1.71 h^{-1} and Q_r= 1628 m^3/h. The results shown in Figure 1 indicate that the observed values and the model predictions follow similar trends suggesting that the modelling

approach provides a reasonable prediction of the likelihood of particle capture by filters. However, for high efficiency filters, the model overestimated particle capture probabilities relative to that measured during the experiments with mean normalized percentage differences between the probabilities calculated during the experiments and the model predictions of 21%, 72%, 63%, 101%, 92%, and 99% for the six size bins considered.

The grey lines in Figure 1 present the model predictions if the measured β and η values are employed. If these two measured parameters are utilized, the model is in much better agreement with the measured filter capture probabilities, particularly for the high efficiency test, suggesting the strong influence of these two parameters in the predictions and the need to accurately estimate them. Table 2 presents the comparison between the β and η values obtained from the literature and those measured during the current experiments. Significant differences between the β and η values measured during our tests and the literature values (utilized in the model) exist, particularly for the β values for the smaller size bins. The deposition loss rate (β) has been reported to vary significantly depending on several factors including the structure of the house, building conditions, and mixing conditions (Nazaroff *et al.*, 1993; Riley *et al.*, 2002). During our particle decay tests to estimate the β values, eight fans and one ceiling fan were operated to increase the mixing and the obtain well-mixed conditions. This high level of mixing likely caused elevated average velocities in the house that could have increased the likelihood of particles to collide against a surface and remain attached to it. This phenomenon is likely to be more important for smaller particles that have lower deposition loss rate and tend to stay longer in air, and may be responsible for the elevated difference in β values between the experiments and the literature values for small particle size bins (Table 2). In Figure 1, we notice a greater difference between the predicted and

measured filter removal fraction for particles in the 0.3 to 3 μm size range, mainly because for that size range, the β values estimated during our tests were much greater than the values assumed in the model based on literature values (Table 2). Better agreement between the model predictions and the experimental observations was evident for particles greater than 3 μm, where the model may simulate effectively what was observed during the experiments. We also observed differences between the filter efficiencies (η) measured during the tests and the literature values used in the model (Waring and Siegel, 2008), particularly for the high efficiency filter. The efficiency values obtained from the literature were for clean new filters and were determined by ASHRAE Standard 52.2 (ASHRAE, 2007). Filters in real systems and conditions may perform differently than that estimated in the Standard 52.2 tests, particularly if they are challenged with particles of a different nature or if bypass occurs.

For low efficiency filters, the model and the experiments are in better agreement, particularly for larger particle sizes ($\geq$ 2 μm), than during the high efficiency tests. In the low efficiency filter scenario we observed mean normalized percentage differences between the probabilities calculated during the experiments and the model predictions of 81%, 89%, 18%, 31%, 19%, and 23% for the six particle size bins considered. The probabilities for the low efficiency filter scenario are relatively constant across the size range, around 0.15, revealing that these filters are not likely to oversample a particle size range and therefore show promise as samplers that are not biased towards a specific particle size.

Table 2. Comparison between literature and measured values for the β and η coefficients

Size bin (μm)	β (h^{-1})			η (-)					
				Low efficiency filter			High efficiency filter		
	Literature	Tests	% difference	Literature	Tests	% difference	Literature	Tests	% difference
0.3-0.5	0.03	1.00	97	0.01	0.13	92	0.43	0.33	30
0.5-1	0.05	1.77	97	0.01	0.12	92	0.66	0.58	14
1-3	0.92	4.01	77	0.08	0.11	27	0.86	0.77	12
3-5	3.37	6.88	51	0.13	0.29	55	0.96	0.90	7
5-7	7.10	9.95	28	0.11	0.06	83	0.98	0.92	7
7-10	14.45	14.3	0.8	0.13	0.07	85	0.98	0.96	2

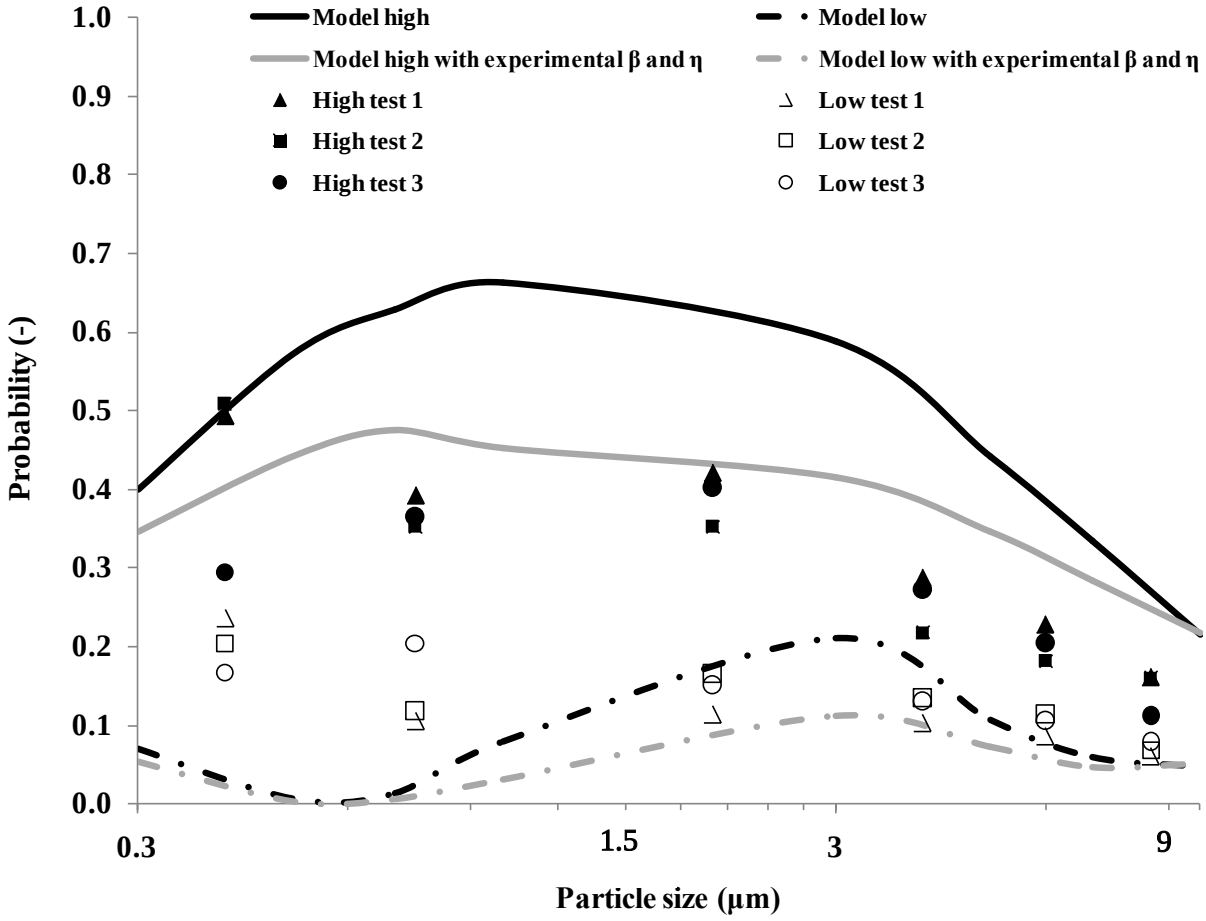

Figure 1. Comparison between model predictions and the observed capture probabilities during the experiments with the low and high efficiency filters.

The variations between the model predictions and the experimental results confirm the complexity of the phenomena and suggest that several factors are important and play a role in the fate of particles in an indoor environment. For instance, even with the high level of mixing present during the tests, the assumption of perfectly well-mixed conditions in the house may not have been met and this will affect the predicted particle concentrations and capture efficiencies. If the well-mixed assumption is not met, there are lower (or greater) particle concentrations near the return and the particles have actually a smaller (or greater) probability of being captured on the filter than what the

model predicts with the assumption of perfectly mixed conditions. Another factor that could have influenced the results is the assumption of constant density across the particle size range. It is possible that larger particles may have greater density because they are more likely to contain crustal material (Seinfeld and Pandis, 1998). The fact that we assumed a lower density for larger particles would lead to an over prediction in the volume (or number) of particles injected, v_i, which would lead to an under prediction in the probability of filter capture, $p_{f,e}$. Moreover, the particles were assumed to be spherical and a characteristic size for each bin was utilized to estimate the volume of the particles measured on that bin. This assumption could have also affected the filter capture probabilities because a different volume of particles could have been injected.

Filter Capture Probabilities

Even with the discrepancies described above, the experiments in the full-scale test house indicate that the modeling approach can be utilized to estimate the likelihood that particles are collected on filters or are removed from the air via other mechanisms such as deposition or exfiltration. Subsequently, in order to evaluate a broader application of filters as samplers, the model was applied to more realistic cases and conditions using typical characteristic times for each removal mechanism reported in the literature. Figure 2 shows the removal probability via different mechanisms for the baseline scenario (MERV 6 filter, $\lambda_r = 1.1\ h^{-1}$ and $\lambda = 0.5\ h^{-1}$). The results indicate that, for a mid-efficiency filter, large particles (> 3 μm) are likely to deposit on surfaces and are unlikely to get captured on filters. The deposition probability increases with size due to the increase in the deposition loss rate (β) for larger particles. Particles in the 0.03-3 μm range are likely to be removed by exfiltration or, if a high efficiency filter is installed, captured on the filter (Figure 3). Particles in this range have a greater residence time in air and, therefore,

have a greater opportunity to be captured by the filter. The filter removal probability increases for ultrafine particles (< 0.1 µm) since the filter efficiency for these particles is greater for all three MERV ratings. Sippola *et al.* (2003) and Zhao *et al.* (2009) reported that large particles (> 1 µm) are likely to deposit on surfaces, while small (submicron) particles tend to be exfiltrated. Figure 2 shows two peaks in the filter capture probabilities, at approximately 0.01 and 3 µm. For the baseline scenario conditions, filtration is about 18 times less likely to capture a 0.1 µm particle before it is removed by exfiltration and 12 times less likely to capture a 5 µm particle before it deposits on a surface. For an HVAC system operating only 22% of the time, particles between 0.03 and 1 µm are most likely to be removed by exfiltration and are less likely to be found in settled dust or in the dust that collects on a mid efficiency filter. From Figure 2, we notice how HVAC filters capture particles over a wide size range and are likely to be effective overall samplers. In contrast, settled dust may be biased toward larger particles that have greater mass, while air samplers may oversample those particles (0.03 - 3 µm range) that have a longer residence time. In addition, although the filter capture probability illustrated in Figure 2 does not seem particularly elevated relative to deposition or exfiltration, it is important to note, despite the higher removal probability for these two mechanisms, it may be more difficult to obtain a representative sample since settled dust and air samples only sample a very small fraction of the total surface or volume in a building at a particular time. In contrast, HVAC filters are typically in place for several weeks and, during this period, a great volume of air is filtered and a significant portion of the filter dust cake can be analyzed easily.

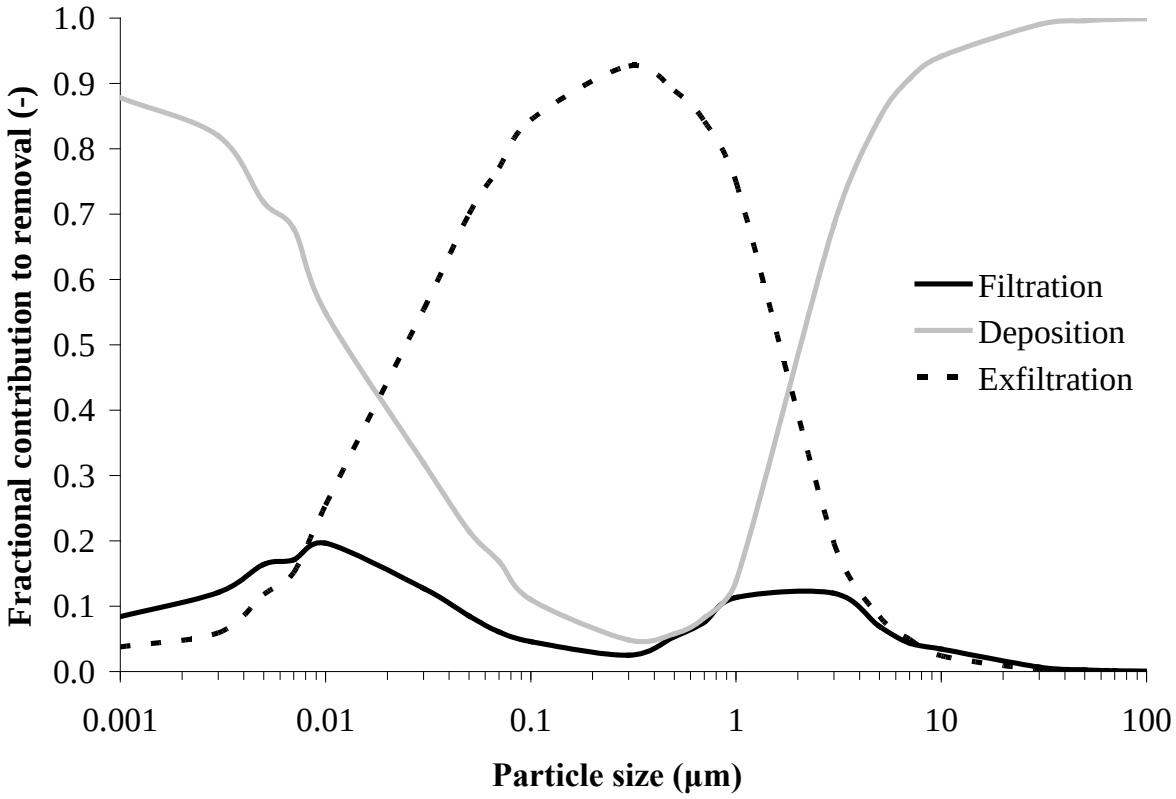

Figure 2. Removal probability curves for filtration, deposition and exfiltration for the baseline scenario (MERV 6, λr=1.1 h^{-1} and λ=0.5 h^{-1}).

Additional results of the analysis are presented in Figures 3, 4, and 5; in each case, one parameter at a time was modified from the baseline scenario. Figure 3 shows that the filter efficiency is a more important variable for sampling particles in the range between 0.03 and 3 µm than for particles below 0.03 µm or above 3 µm. These results are due to the fact that the HVAC filter efficiency is particle-size dependent especially in the range from 0.01 to 3 µm (Hanley *et al.*, 1994). The high efficiency filter probability curve has a minimum around 0.1 µm due to the reduced efficiency of the filter for particles around this dimension. Low and mid efficiency filters have similar probabilities for a wide range of particles, although a greater variation is observed in the range

between 0.3 and 10 µm. A MERV 11 filter is approximately 9 times more likely to capture a 0.5 µm particle than a MERV 6 filter and about 150 times more likely than a MERV <5 filter. If a high efficiency filter is used, there is an elevated probability of capturing particles in the 0.3 – 3 µm and 0.005 – 0.03 µm ranges. In the size range of 0.3 – 3 µm, the model predicts that more than one third of the particles should be captured on a high efficiency filter. As a consequence, high efficiency filters are likely to be reasonable samplers for particles in these size ranges. For larger particles (> 3 µm), deposition onto surfaces is the dominant removal mechanism and filters are less likely to be good samplers.

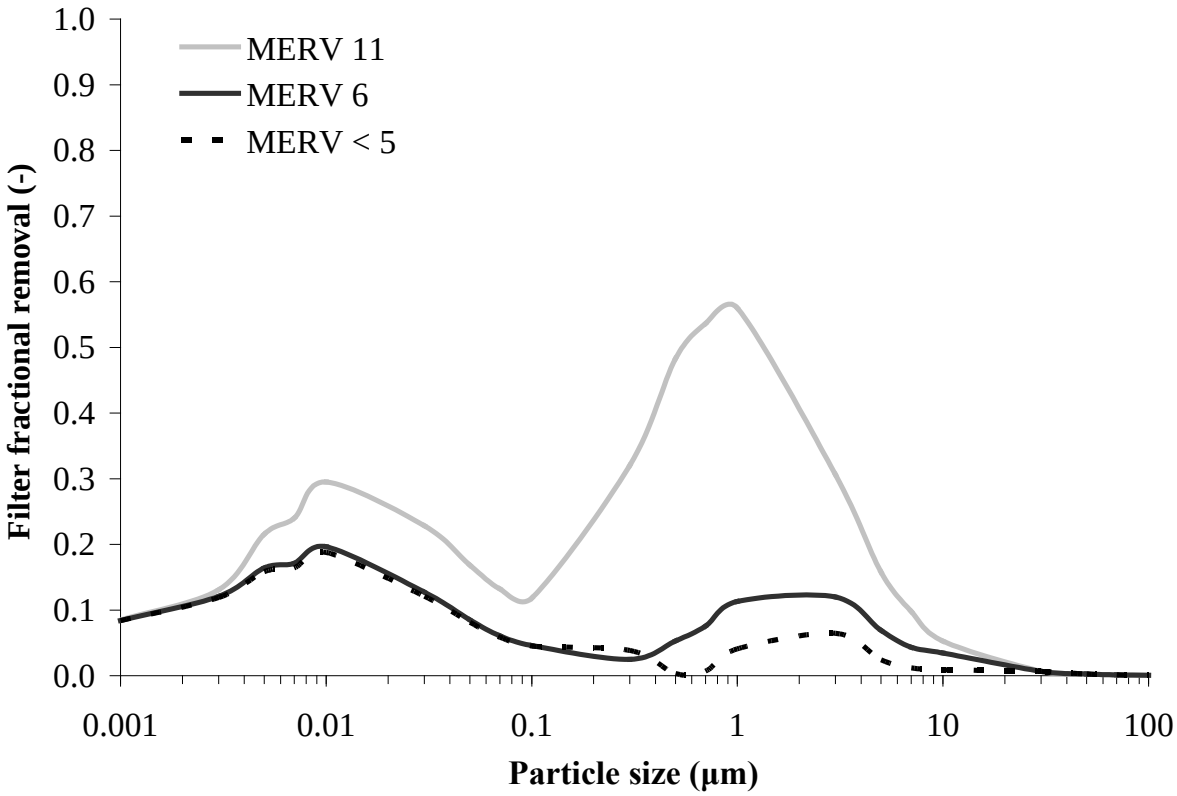

Figure 3. Filter capture probability curves for different filter efficiency scenarios.

Figure 4 illustrates the influence of the HVAC system duty cycle on the filter capture probability curve. The profiles for the two λ_r values investigated follow similar

patterns, with the probability for removal via filtration for normal (cycling) use reduced approximately by the duty cycle fraction (0.22) relative to the continuous operation case. The greatest difference between the two duty cycle scenarios is evident for particle sizes that are more likely to be captured on the filter, 0.01 and 3 µm. The results suggest that, if a mid efficiency filter is used, the HVAC system would need to operate with an elevated duty cycle in order for the filter to be an effective sampler. However, high efficiency filters with elevated recirculation air exchange rates (> 5.2 h^{-1}) are particularly effective, with more than 30% capture probability up to 7 µm and often above 60% (data not shown). Filters are more effective particle samplers if they have either high removal efficiency or if the HVAC system has an elevated air recirculation rate. If both of these two conditions are met filters, the filters are more likely than air or settled dust samples to capture particles in a wide size range. In particular, for these conditions, the particle capture probabilities onto the filter are predicted to be as high as 85% for particles around 1 µm.

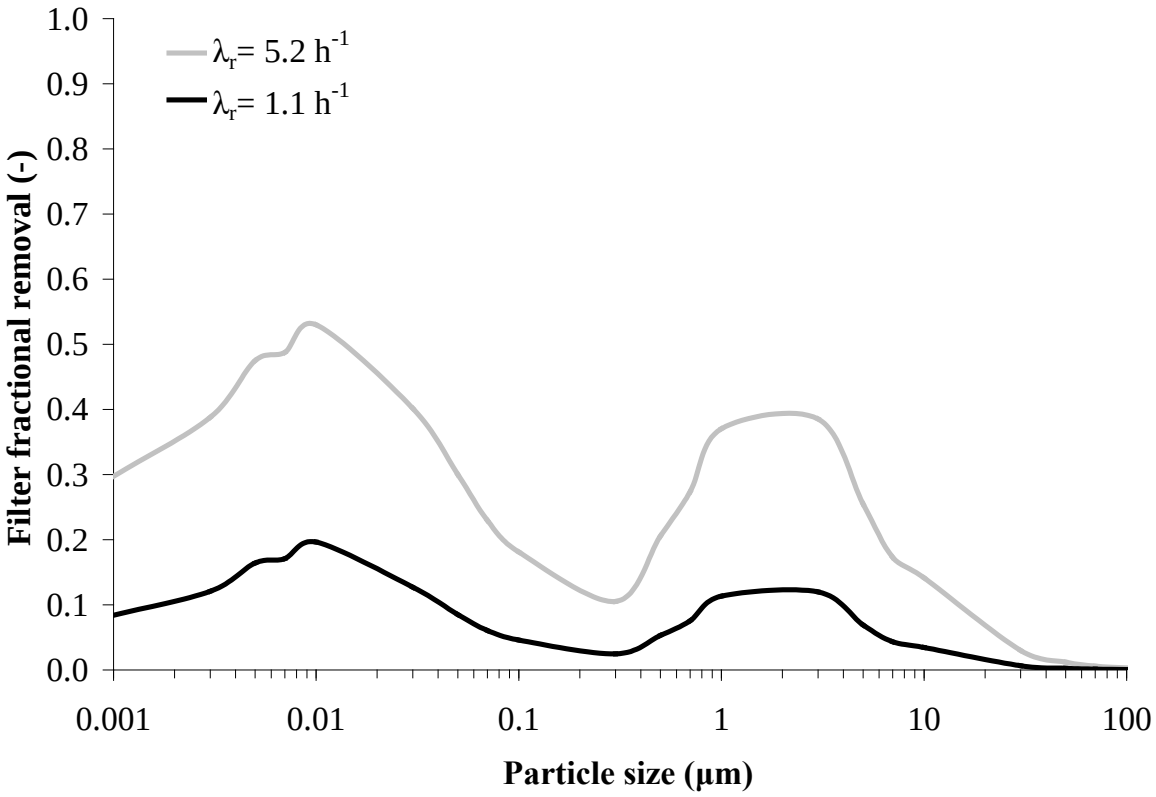

Figure 4. Filter capture probability curves for different air recirculation rate scenarios.

Figure 5 presents the filter probability curves for residences with varying tightness. Abt *el al.* (2000) found that air exchange rate has a significant effect on particle removal. As evident in Figure 5, this parameter does have an influence on the probability of particle capture on the filters; however, this parameter does not seem to be as important as the filter efficiency or the λ_r with similar patterns and capture probability among the three scenarios investigated. For instance, the difference in filter capture probability between a residence with $\lambda = 0.2$ and one with $1.3\ h^{-1}$ is, typically, below 10%. As a consequence, filters could potentially be used as samplers independently of the tightness of the residences investigated. This is an important consideration since the current trend is to move toward tighter and more energy efficient buildings.

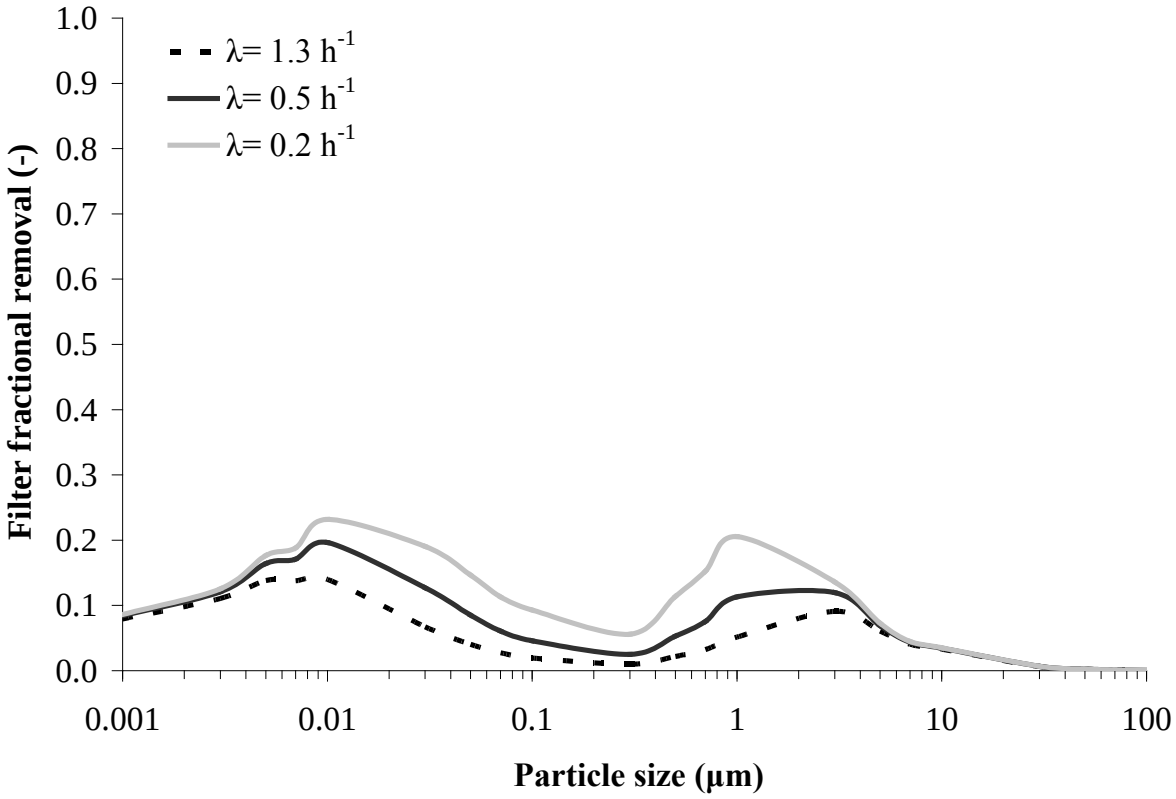

Figure 5. Filter capture probability curves for different air exchange rate scenarios.

This analysis suggests that HVAC filters may be used as passive samplers that are in place for long periods of time and can overcome the short-term sampling limitations of traditional air samplers. In particular, HVAC filters capture particles over a wide size range and can be considered effective overall samplers. In contrast, as can be seen in Figure 2, settled dust samples are biased toward larger particles, while conventional air samples may oversample those particle sizes that are not removed effectively by other mechanisms and tend to stay longer in the air (0.03 - 3 µm range). The best way to increase the probability that a broader size range of indoor particles will be captured by the HVAC filter is to increase the filter efficiency. This variable has a greater predicted effect than increasing the λ_r or decreasing the λ. High efficiency filters, in particular, could develop into a less intrusive and effective way to obtain information regarding the

indoor contamination in homes. This information could be integrated with conventional indoor air sampling strategies or, depending on the data being sought, it may provide an alternative, more efficient mechanism for collecting samples during large-scale investigations of multiple residences. However, as with all sampling methodologies, using HVAC filters as samplers has limitations that must be considered when interpreting the data collected. For instance, the influence of filter location relative to potential particle generation sources is a variable that should be considered and investigated further. In an investigation related to the effectiveness of portable air cleaners, Novoselac and Siegel (2009) reported the importance of device location with respect to the particle source. Similarly, we expect that the locations of the filter and return vent will be important factors that affect particle capture on the HVAC filter.

An additional limitation of using HVAC filters to sample the indoor environment is that in certain geographical regions or among specific socioeconomic groups, a significant fraction of the residences may not have a centralized air conditioning system, or the system is not used for certain seasons and, therefore, filters are not a sampling option. Even for the buildings that have a centralized air-conditioning system with built-in filtration, the occupants have significant control over several important factors including the efficiency of the installed filter and the duty cycle of the HVAC system. Filters have a possibility of collecting particles only when they are operated and, as a consequence, when there is limited need for conditioning, filters are unlikely to be effective samplers. Additionally, even if HVAC systems have elevated air recirculation rates, the use of filters are samplers will be affected by when the system is operated relative to when the contamination occurs and therefore the importance of HVAC system cycling may required further investigation. Filters located in systems with elevated return side leakages may not be representative samplers of indoor particle-bound

contaminants, particularly if the ducts and system are located in the unconditioned space. Filters capture particles and, therefore, can only be used for investigating particle-bound contaminant concentrations. Specifically, they are effective samplers for those particles that are not likely to deposit on surfaces and tend to stay suspended in air, thus increasing their chances to be captured on the filter. Finally, another factor to be considered is how to obtain a representative sample of what is captured on the filter. This is an important aspect that requires a careful sampling procedure because certain particles may tend to stay attached to the filter fibers, leading to biases in the particles that are actually analyzed.

CONCLUSIONS

The effectiveness of HVAC filters as passive samplers was investigated for a range of typical residential scenarios. A model based on the characteristic time of each process was applied to evaluate the importance of the main removal mechanisms for indoor airborne particles. Experiments in a full-scale test house corroborated the validity of the model, although some discrepancies exist between the experimental results and the model predictions, confirming the complexity of the phenomena involved. Typical values from the literature were used to investigate the influence that filter efficiency, air recirculation rate and air exchange rate have on the predicted filter capture probability for a range of particle sizes with the objective of evaluating the use of HVAC filters as long-term samplers. Large particles are likely to deposit, while particles in the 0.03-3 μm range stay suspended in air longer. The fate of particles with different sizes also has implications on the particles likely to be collected by conventional air and settled dust sampling techniques. Our scaling analysis indicates that filter efficiency is more important parameter than air recirculation rate or air exchange rate. High efficiency

filters have an elevated probability of capturing a wide range of particle sizes and could potentially develop into an attractive sampling alternative especially if the recirculation air exchange rate is elevated and the HVAC system is operated frequently. However, filters may not be good samplers for large particles or if the system operates with a reduced duty cycle. Other critical parameters including the location of the return vent and filter with respect to the emission source, the zoning of the building and the frequency of the HVAC cycles, could also play a significant role and should be investigated further.

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Appendix D

DNA-Based Methodology

There are several steps in the microbial community analysis. First the DNA needs to be extracted, then amplified, cloned and finally sequenced. Details are provided below.

DNA EXTRACTIONS

DNA extraction is the most critical step. To amplify the DNA, it is necessary to extract a sufficient amount of DNA while minimizing the concentration of inhibitors that can prevent amplification. Several different DNA extraction protocols were investigated for the HVAC dust samples as described below. Based on the results of these preliminary experiments, a modified Power Soil DNA extraction procedure was ultimately used to extract the DNA from the dust and air samples collected in the study.

Direct Extraction. In this set of experiments, the author attempted to extract DNA from the microorganisms directly from the dust by immersing 0.5g of dust into the extraction tubes and the solutions provided by the Power Soil DNA kit. The quantity of inhibitors (visible on the peaks of the Nanodrop) present in the dust prevented the PCR amplification from taking place. The Wizard DNA Clean-up kit was subsequently used to try to purify the DNA extracted. Similarly, an ethanol precipitation step (Frank, 1997) was utilized to try to improve DNA recovery. In both cases, the DNA obtained could not be amplified via PCR. One of the main challenges is to obtain a balance between a sufficient amount of DNA and good purity of the DNA extracted. During every clean up, some DNA is lost, therefore, it is important to start from elevated DNA concentrations to account for potential losses during the purification steps. In an attempt to increase the DNA yield, thaw and freeze cycles (3 cycles) for a range of times and temperatures was attempted but this protocol did not improve the results. The problem was partially overcome by using a MP FastPrep-24 (QBiogene) to lyse the cells instead of using a vortexing step. The use of this device increased the DNA yield. However, DNA

amplification still did not occur due to the inhibition caused by substances present in the dust. The presence of inhibitors in the dust was verified during tests in which positive controls (*Pseudomonas putida*) were spiked with different aliquots of DNA extracted from dust samples. In these tests, the inhibitors present at elevated concentrations in the dust prevented the amplification of the positive control, while the positive controls with no dust DNA spike were amplified.

Separation Followed by Extraction. To work around the inhibitor problems mentioned above, the author decided to separate the microorganisms from the particles before extracting DNA from them. This was based on the protocols used by Colorado University at Boulder researchers (Dr. Norm Pace's lab group) that we have been collaborating with as well as the methodology utilized by Tringe *et al.* (2008). As discussed in the main text of the executive summary and in Appendix B, there were two filtration steps: in the first filtration step, the large particles were separated from small particles and microorganisms; in the second step, the microorganisms were separated from the liquid and captured on the filter. This methodology proved much more effective at removing inhibitors while yielding elevated DNA concentrations.

PCR AMPLIFICATION

After the DNA was extracted from microorganisms, the PCR reaction could be performed. Initially, there were problems amplifying the DNA extract due to the inhibition problems mentioned above and due to the reduced number of cycles (i.e., 16-20 cycles) at which the PCR reactions were performed. However, for cloning and sequencing techniques, a greater number of PCR cycles are typically employed and once the number of cycles was increased to 35, amplifications were able to occur. Another

important parameter to optimize was the aliquot of DNA extract to use in every PCR reaction. The author performed tests with different aliquots and observed the best amplification for PCR reactions was achievable with 2µl aliquots.

GEL PURIFICATION AND CLONING

Once the DNA was extracted and amplified, it was important to remove the DNA fragments that belonged to unused primers due to partial amplification. Therefore, a gel extraction step was employed using a QIAquick Gel Extraction Kit. In this step, it is critical to perform the gel extraction under the UV lamp very quickly and to use fresh and effective ethidium bromide to enhance the visibility of the band.

Subsequently, the DNA fragments could be cloned using the TOPO TA cloning kit for sequencing. For this step, there were several important aspects to keep in mind during the procedure. First, it was important to increase the incubation time to the maximum recommended time for all the steps. In addition, the competent cells had to be thawed in ice and after the temperature shock step in the water bath, they needed to be transferred immediately into ice. Finally, the volume of PCR product introduced into the cloning reaction is a key parameter that must be considered. The author obtained the best results using an elevated volume (i.e., 4µl), but always performed two cloning reactions using different volumes; the the reaction that yielded the greatest number of colonies on the plate, ideally at least one hundred colonies for each plate was used. Subsequently, approximately one hundred clones were picked, isolated, and the plasmid extracted following the protocol described in the Fast Plasmid Mini Kit 5 Prime Inc. (Gatheirburg, MD). Finally, after adjusting the concentration to 50 ng/µl by dilution, the clones were sent to sequencing on ABI 3730 DNA analyzer (Applied Biosystems, Foster City, CA).

Appendix E

METRICS AND PARAMETERS UTILIZED

The Weighted Unifrac method was chosen for a variety of reasons. This parameter has been used successfully in a variety of recent indoor (Rintala *et al.*, 2008; Täubel *et al.*, 2009) and outdoor (Fierer *et al.*, 2008) studies. The main advantage of the Unifrac analysis is that it compares the structure of the communities and not just the membership of each community; in this way, it can provide a statistical comparison between communities to evaluate the hypothesis that a given pair of microbial communities is statistically different. This Unifrac method analysis does not take into account the diversity in terms of the different species present in communities, but only assesses whether the clones are phylogenetically distant or similar and does not explain the nature of the differences. The Unifrac analysis relies on phylogenetic information, while compositional graphs rely on a group classification and does not characterize the community. Therefore, it could be possible to have members of the same phylum, different on a phylogenetic level. At the same time, it is possible also to have the opposite scenario with species of different groups, being extremely different or quite similar on a phylogenetic level. The Unifrac does not assess this. For all the reasons above, it is useful to couple a statistically test, like the Unifrac analysis to a more conventional assessment of the diversity such as the classification of the microbial community compositions, as was done in the current study.

The next two figures present the rarefaction curves for bacterial and fungal clones. In both cases, the communities were not completely characterized, but the curves were starting to flatten out suggesting that the predicted number of ribotypes should be not too far from what detected in the clones libraries.

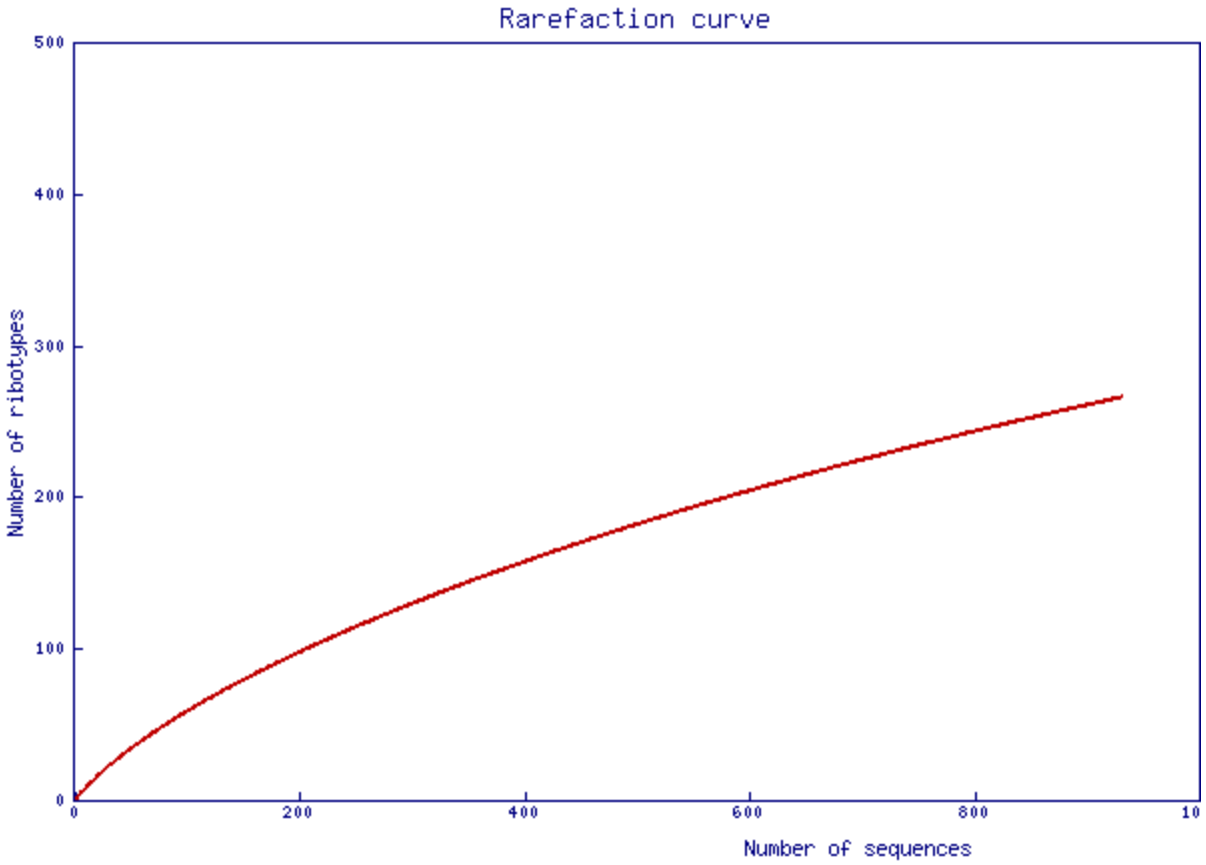


Figure 1. Rarefaction curve for bacterial clones

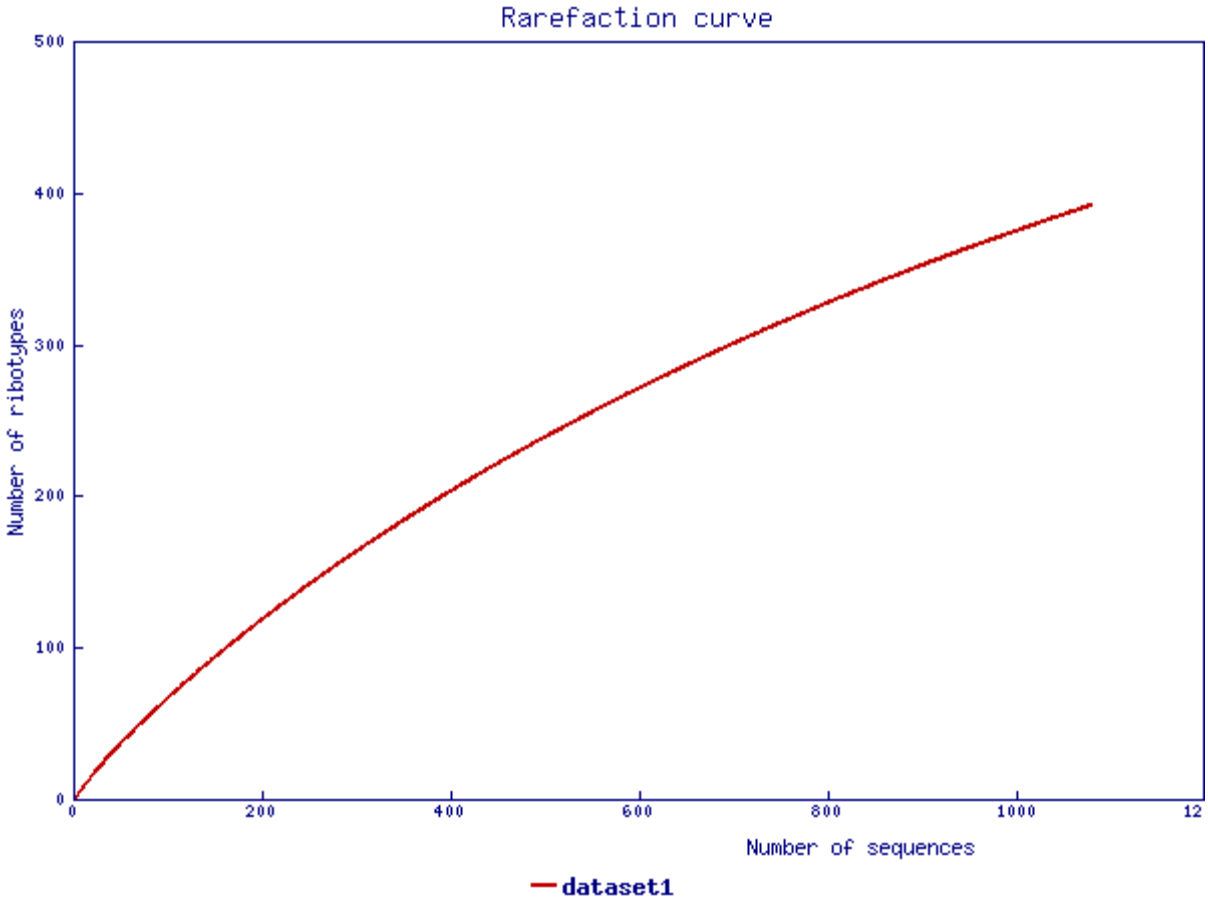
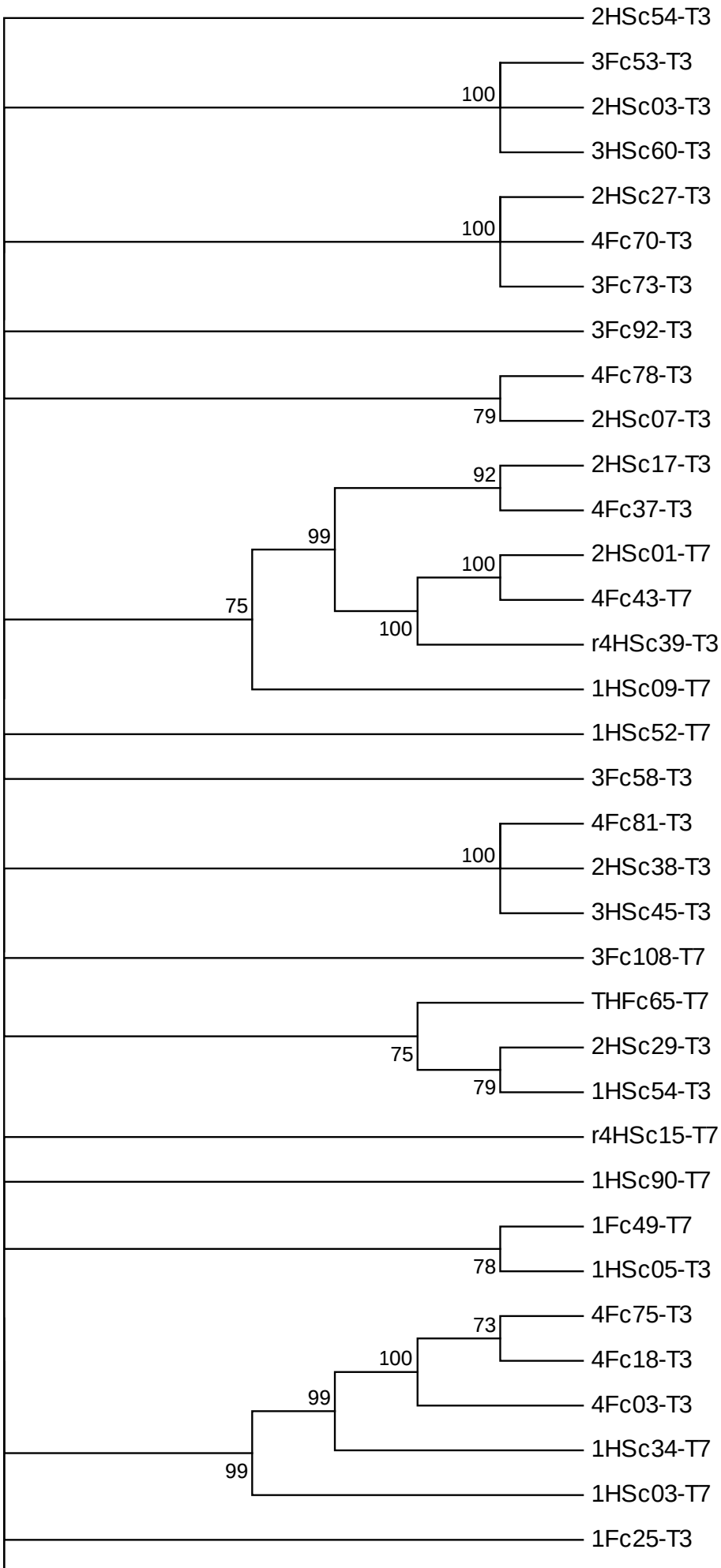


Figure 2. Rarefaction curve for fungal clones.

BACTERIA PHYLOGENETIC TREE

The first symbol represents the site number, therefore the Numbers 1 (Site 2), 2 (site 7), 3 (Site 6), and 4 (Site 5) represent the clones encountered in the residences, while TH represents the clones found in the test house. Following the building identifier, the next letter represent the sampling location, therefore F=filter, HS=high surface. Since air samples were only collected in the test house, the clones that start with IT or OT represent the clones found in the composited indoor and outdoor air samples in the test house. The number following the sampling descriptor represents the clone (c) number.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100
2HSc03-T3
3HSc60-T3
2HSc27-T3
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3Fc73-T3
3Fc92-T3
4Fc78-T3
79
2HSc07-T3
92
2HSc17-T3
4Fc37-T3
99
2HSc01-T7
100
4Fc43-T7
75
100
r4HSc39-T3
1HSc09-T7
1HSc52-T7
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4Fc81-T3
100
2HSc38-T3
3HSc45-T3
3Fc108-T7
THFc65-T7
75
2HSc29-T3
79
1HSc54-T3
r4HSc15-T7
1HSc90-T7
1Fc49-T7
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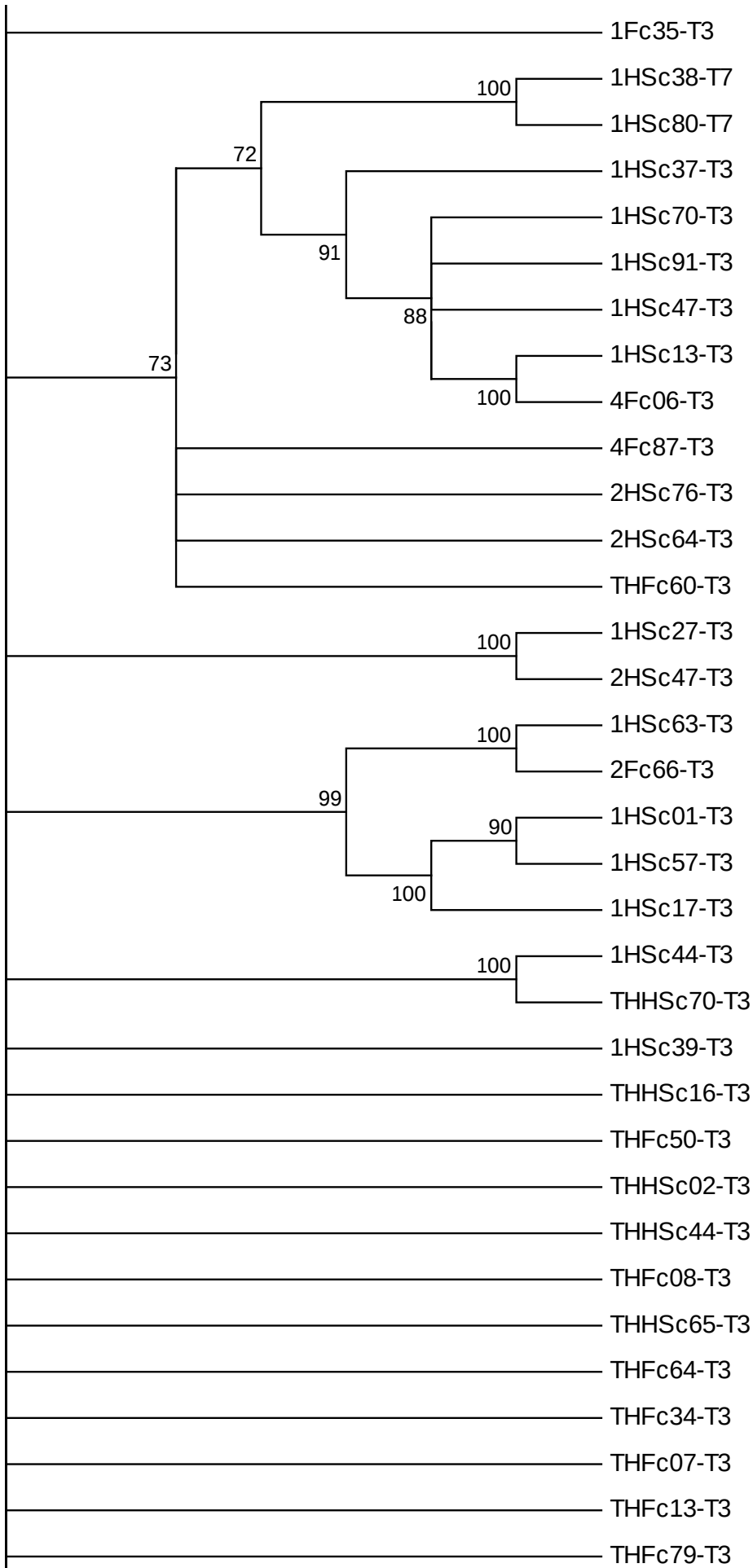
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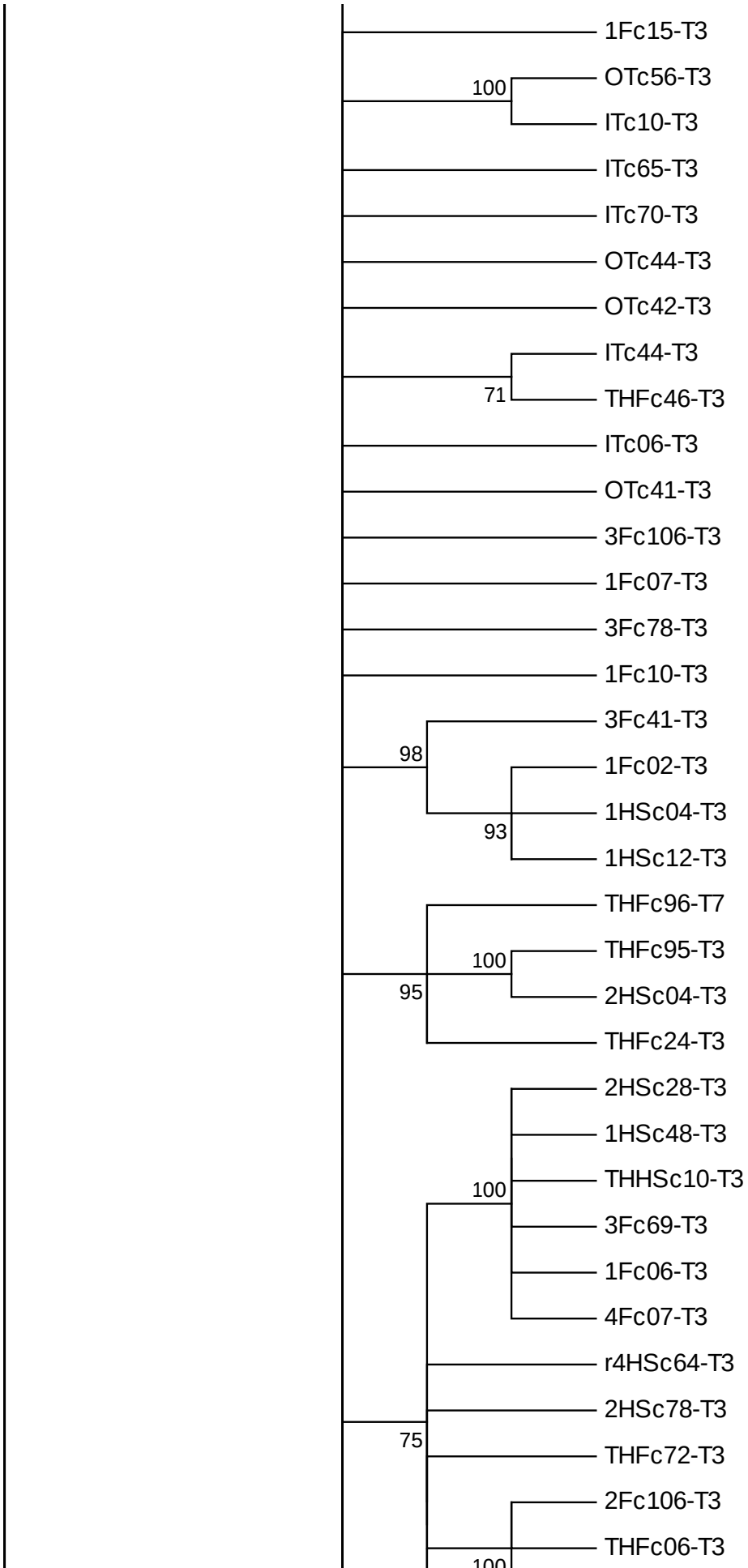
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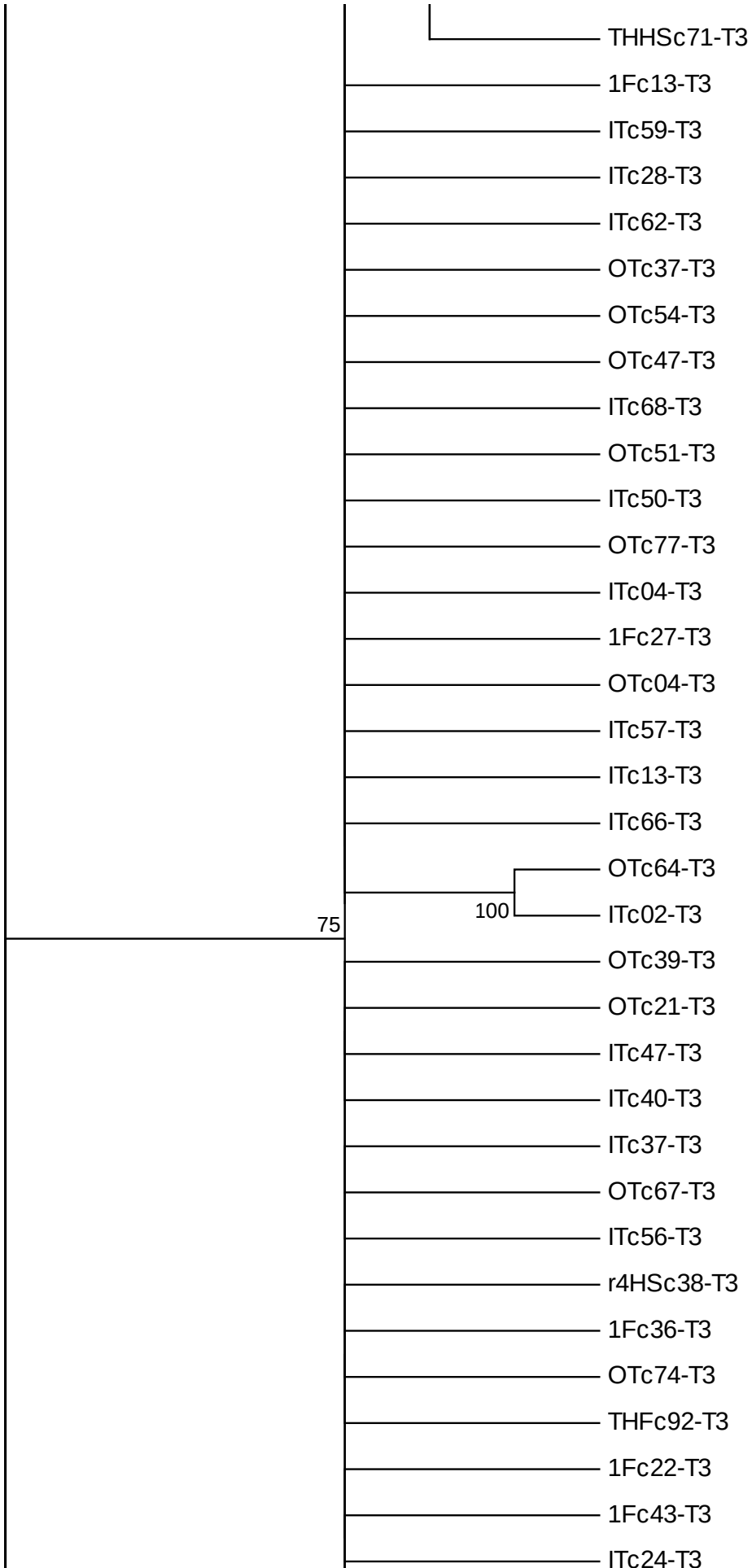
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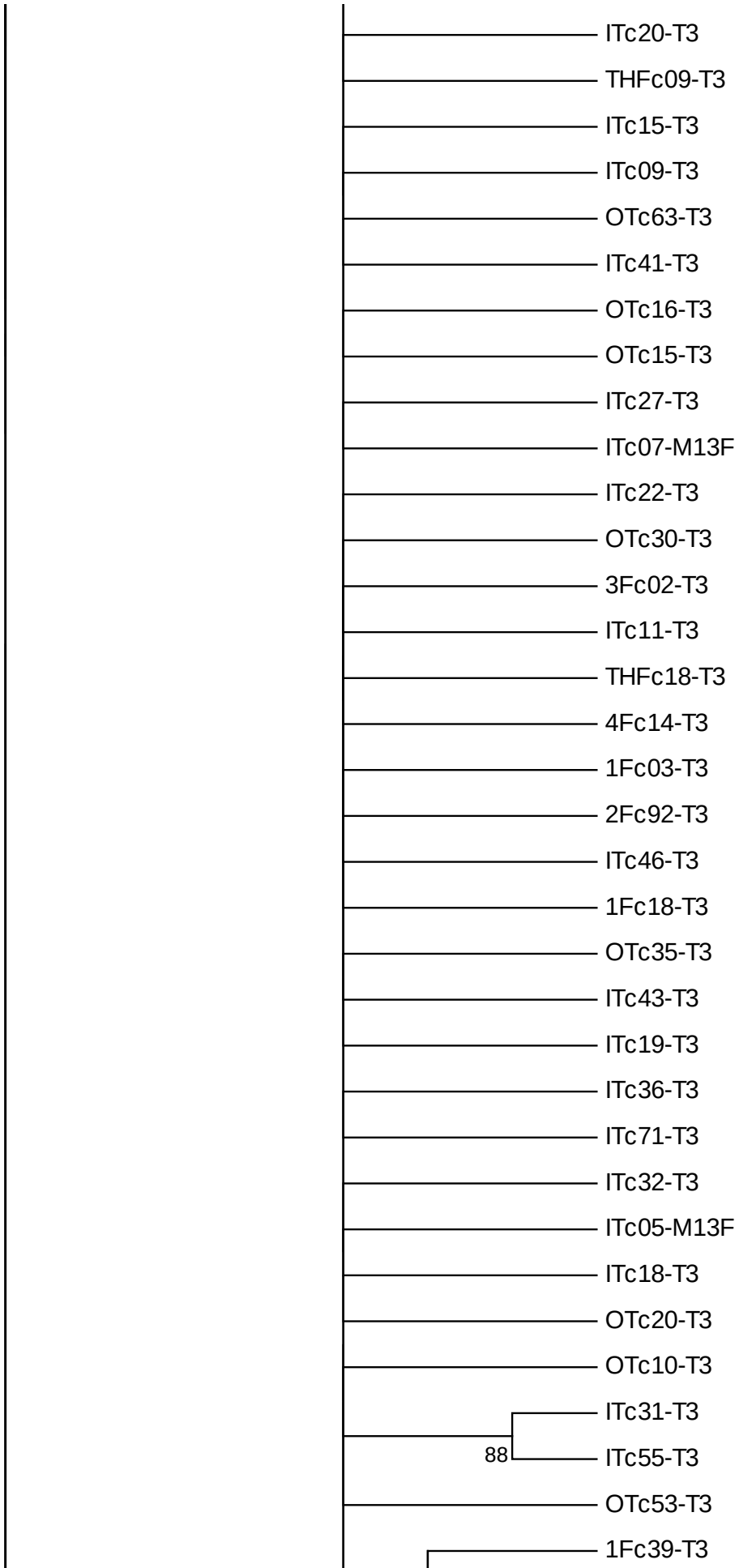
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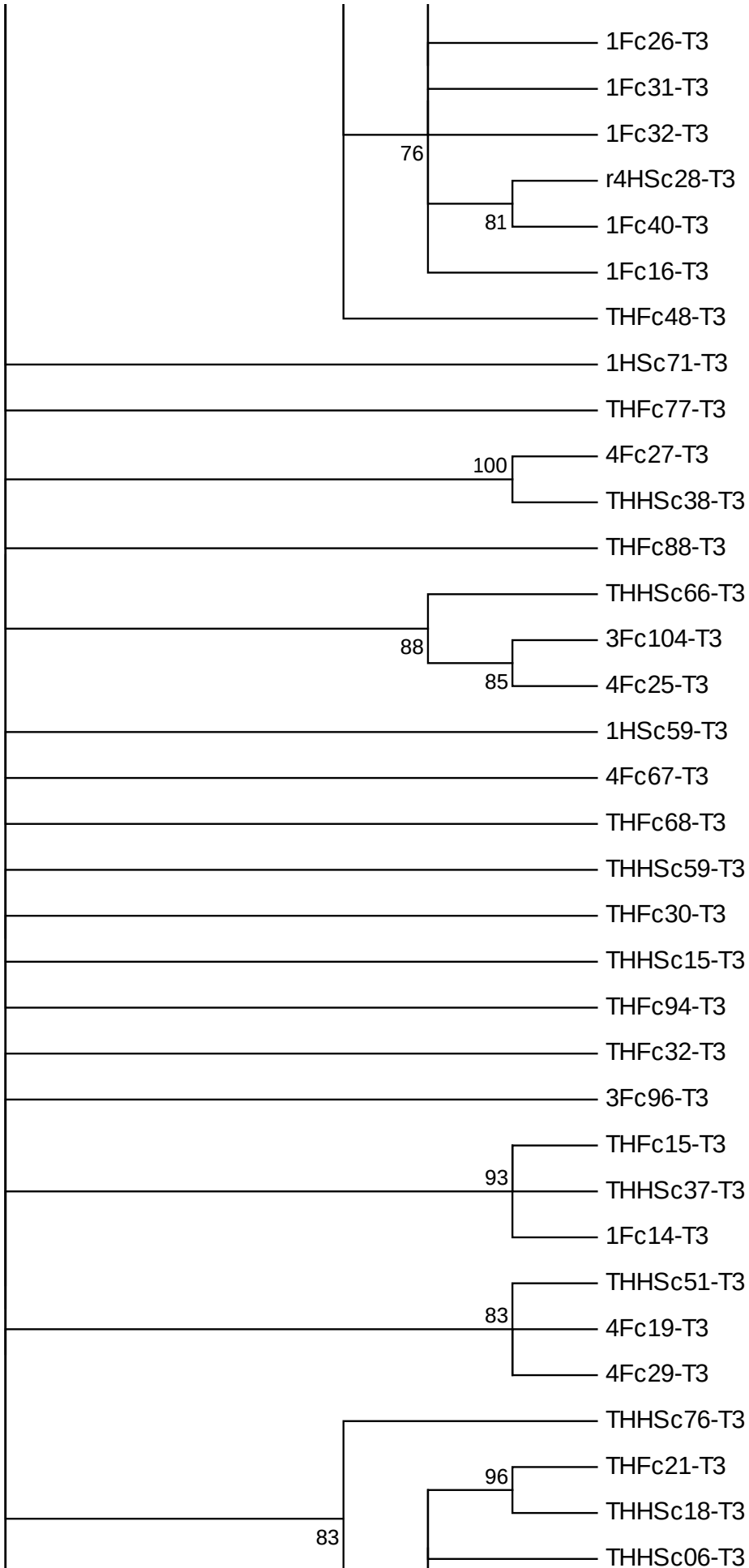
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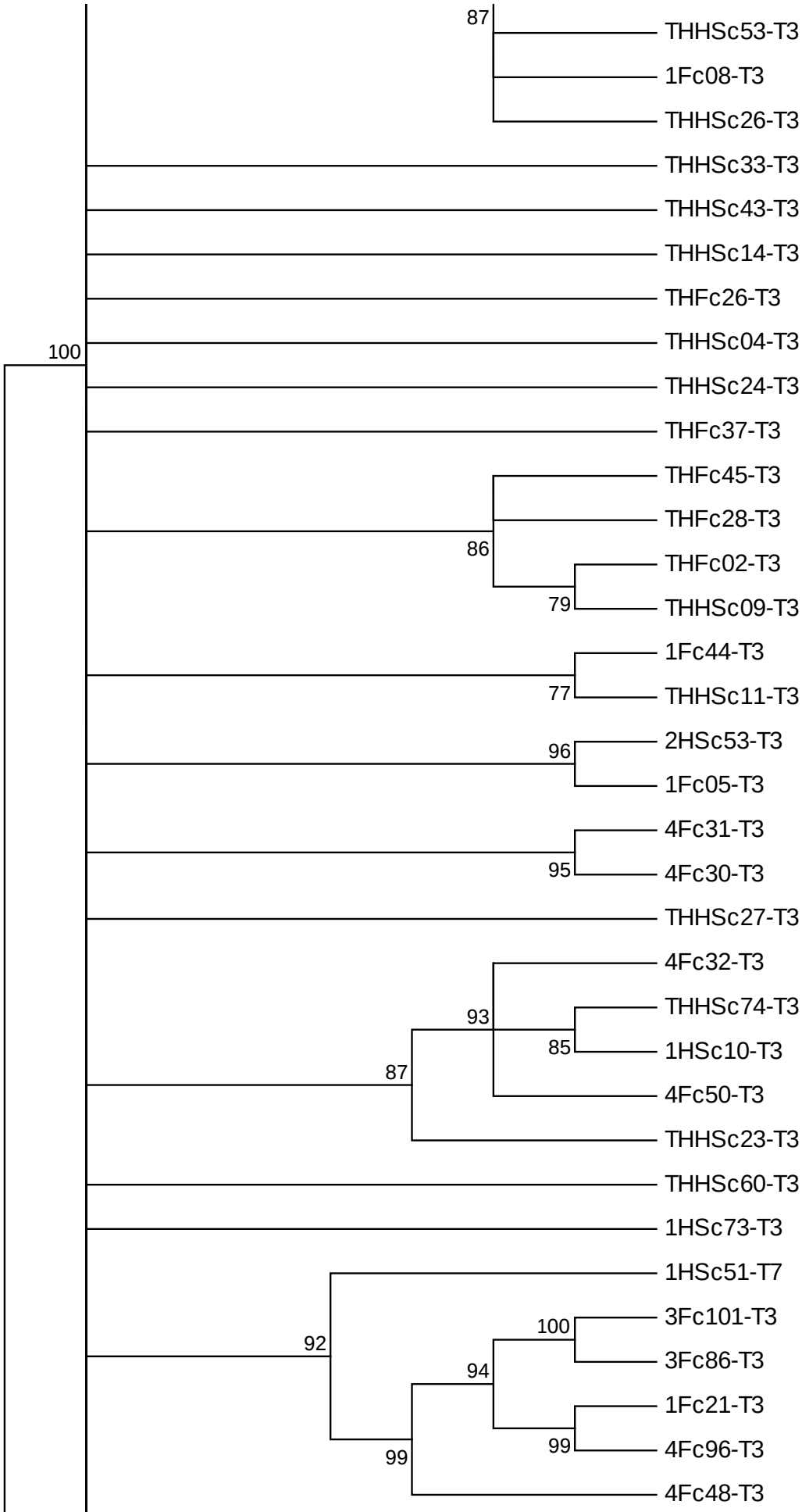
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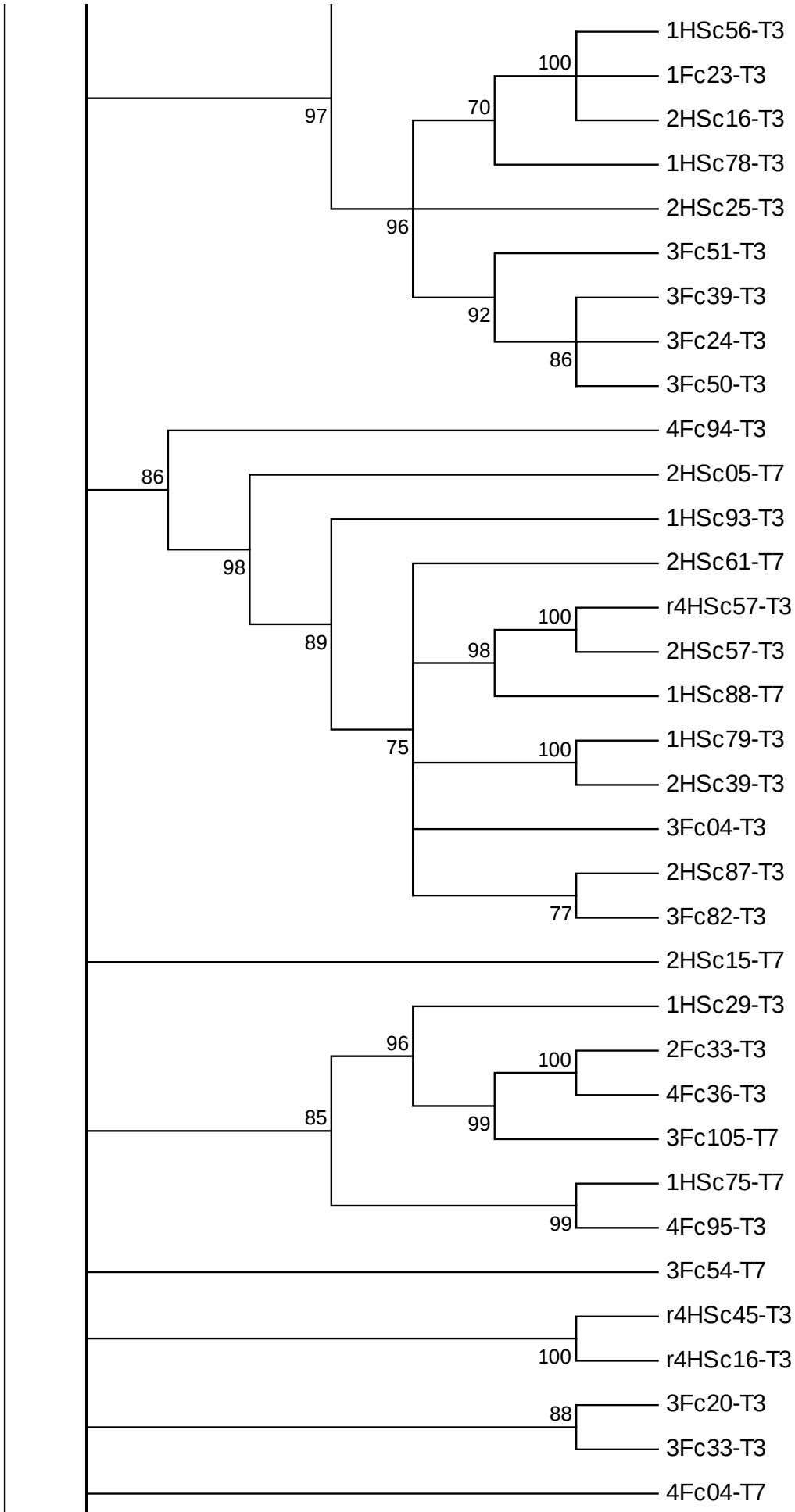
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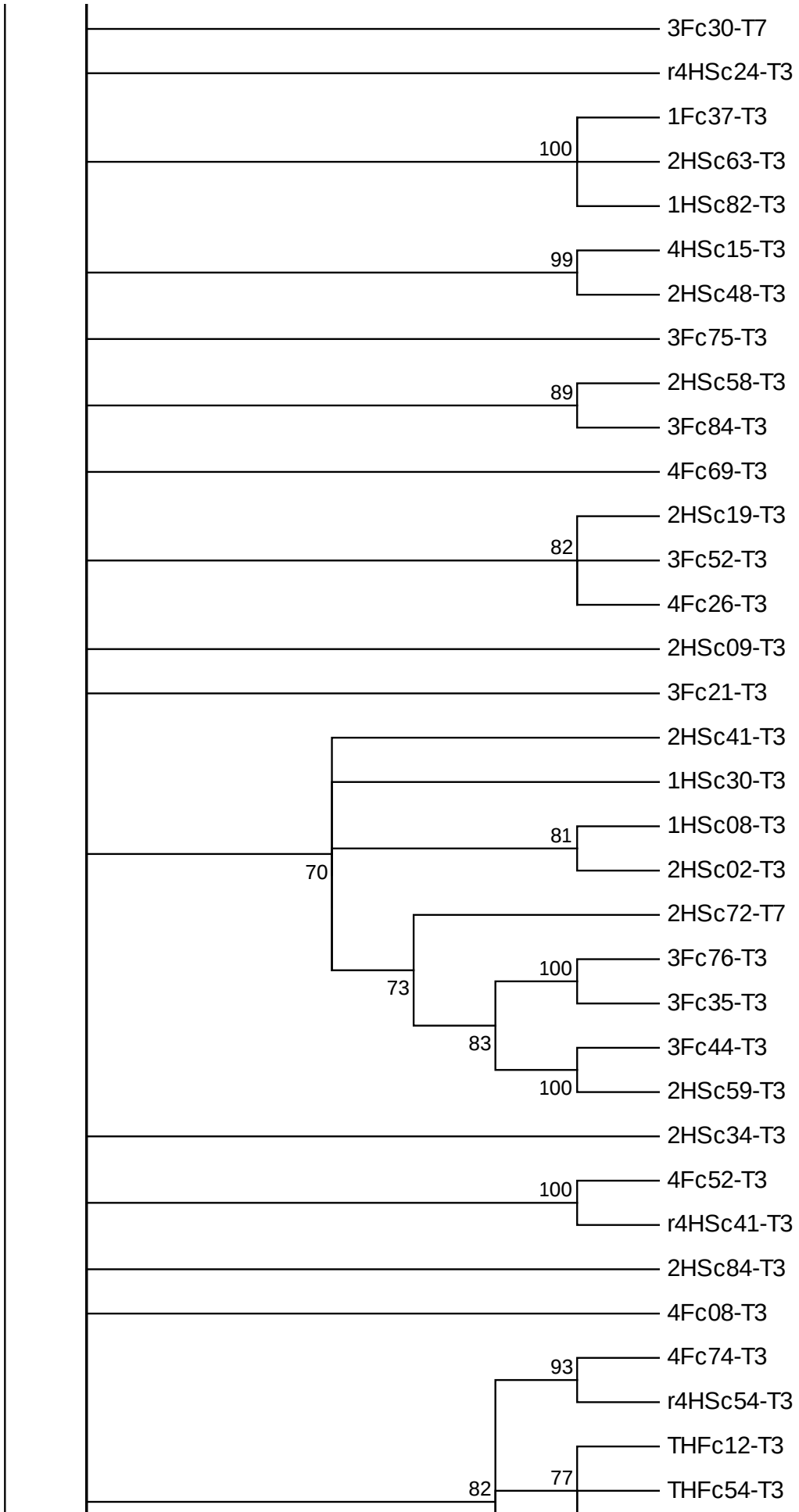
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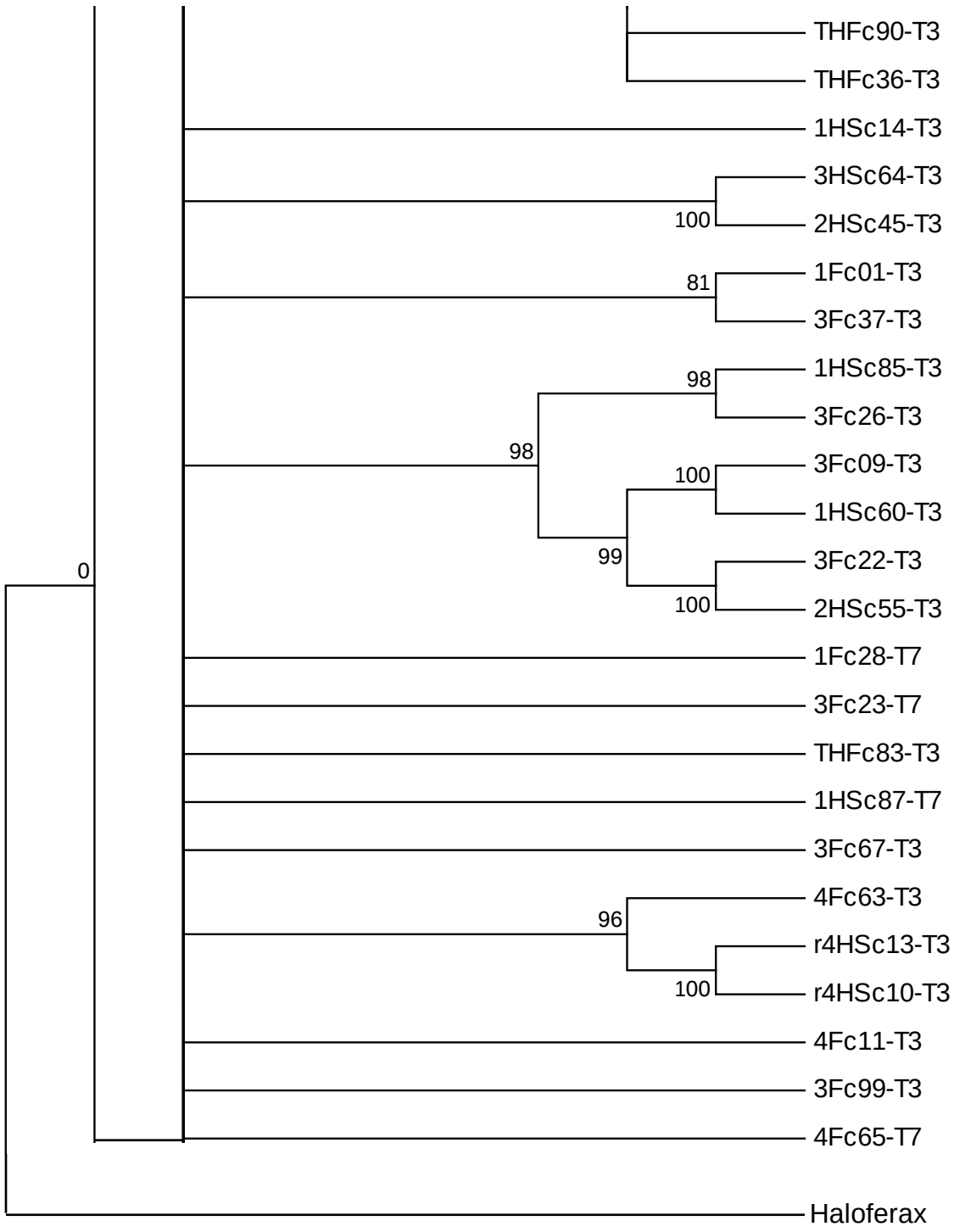
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Haloferax

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Vita

Federico Noris obtained his Bachelor's and Master's degrees in Environmental Engineering from Politecnico di Milano, Italy in 2003 and 2006, respectively. During the final year of both degrees, he came to The University of Texas at Austin to work with Dr. Kerry Kinney on vapor phase biofilter and bioaerosol emissions released from these devices. Subsequently, Mr. Noris entered the Graduate School at The University of Texas at Austin and became a doctoral student in the Department of Civil, Architectural, and Environmental Engineering working with Dr. Kerry Kinney and Dr. Jeffrey Siegel on the evaluation of HVAC filters as a sampling mechanism of the indoor environment.

Permanent address: fedenoris@gmail.com

This manuscript was typed by Federico Noris.