ASSESSMENT OF REGIONAL FUNGAL CONCENTRATIONS AND DIVERSITY AND THEIR POSSIBLE ASSOCIATION WITH SELF-REPORTED HEALTH EFFECTS AMONG A NATIONAL SAMPLE OF OFFICE BUILDING OCCUPANTS IN THE UNITED STATES

by

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To my parents, Arden and Marilyn

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ABSTRACT

Data from the Environmental Protection Agency's Building Assessment and Survey Evaluation (BASE) study was analyzed for culturable fungi detected in air samples collected from 100 office buildings located among ten climate regions in the United States. Fungi identified and quantified in the study were evaluated in indoor and outdoor environments. Evenness of species for both summer and winter, and the diversity and similarity indices of species were calculated between climate region groups in order to observe potential climate-based differences in the fungal microbiome. Respiratory and neurological health symptoms of study building occupants (n = 4,326) were self-reported by questionnaire, and were analyzed in order to assess seasonal and climate differences.

PUBLIC ABSTRACT

Fungi have been implicated as causes of health problems among people, ranging from mild symptoms such as coughs or headaches, to more severe conditions such as allergic responses and infections. Since there are no government standards that indicate what fungal concentrations may be harmful if detected inside a building, it is of interest to study the types of fungi that are present in indoor environments in order to gain insights into their potential source, and to determine whether there are differences in the concentrations of fungi detected across climates that have differing temperatures and humidity. These factors, when compared with reported health effects of building occupants, will be helpful in understanding the relationship between the presence of fungi and peoples' health. This project examines concentrations and types of fungi found in one hundred office buildings across ten climate zones in the United States, from data collected in the Environmental Health Agency's Building Assessment and Survey Evaluation (BASE) study, and further examines the differences (and similarity) of fungal genera detected in winter and summer. The study also explored potential associations between self-reported adverse health effects of building occupants and a cross-sectional sampling of fungal concentrations.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: INTRODUCTION

BACKGROUND INFORMATION

Fungi comprise a kingdom of eukaryotic organisms. They are heterotrophic (i.e., lack chlorophyll), having a nucleus surrounded by a membrane, and cell walls consisting of glucan or chitin. There are well over 100,000 described fungal species (de Hoog, et al., 2000). The requirements for fungal growth include fungal spores, a food source, appropriate temperatures, and sufficient moisture. Changes (e.g., increased moisture) in any of these critical requirements can substantially modify the number and diversity of fungi both in and outside the built environment.

Fungi and Climate Change

The 2011 Institute of Medicine's report, "*Climate Change, the Indoor Environment, and Health*", concluded that climate change may degrade existing indoor environmental problems and create new ones such as deterioration of the building envelope resulting in water intrusion that encourages the growth of fungi (IOM, 2011). In describing climate change impacts in the United States, in the Third National Climate Assessment, Luber et al. (2014) stated that extreme rainfall and rising temperatures can "foster indoor air quality problems, including the growth of indoor fungi and molds, with increases in respiratory and asthma-related conditions." To support their statement, the authors cited a meta-analysis of 33 studies that examined the association of indoor dampness/mold contamination in the home and adverse health effects (Fisk et al., 2007). The findings from the meta-analysis suggested that building dampness and mold were associated with a 30–50% increase in a variety of respiratory-related health outcomes examined (i.e., wheeze, upper respiratory tract symptoms, current asthma, ever been diagnosed with asthma).

The Institute of Medicine's report (IOM, 2011) also highlighted the need to collect data that could be used in the future to make better-informed decisions to reduce the potential health effects caused by climate induced indoor environmental conditions. Surveys of fungi concentrations and diversity in "non-sick" office buildings are limited in number (USEPA, 1994; Gaskin et al., 2012; Taylor et al., 2014). The most comprehensive survey performed globally to provide normative data on indoor air quality parameters, including fungi, in typical office buildings was the U. S. EPA's Building Assessment Survey and Evaluation Study.

BASE Study Overview

From 1994-1998, the US Environmental Protection Agency (EPA) conducted the Building Assessment Survey and Evaluation (BASE) study to provide insight into building stock and indoor air quality (IAQ)-related parameters and to fill a void in previously unknown baseline data among office buildings in the United States (USEPA, 1994). Prior to this time, the assessment of IAQ and health effects in buildings was largely focused on "sick" buildings which, while important, did not present a comprehensive representation of conditions within the building stock in the United States. It was apparent that the need to identify and assess representative (i.e., typical) building conditions, as well as worker comfort and health, was necessary in order to develop informed policy with regard to potential need for future intervention or remediation. Cross-sectional in design, the BASE Study gathered data from 100 randomly-selected office buildings and characterized environmental parameters and occupant perceptions associated with indoor air quality. The study was designed to establish baseline data for public and commercial buildings selected, without regard to IAQ complaints, among a range of ten identified geographic climatic regions based on air conditioning and heating use and humidity- (ASHRAE, 1989). In each of the buildings included in the study, data were collected

in one-week study periods using sampling and assessment methodologies and a survey schedule defined in the EPA's protocol titled "*A Standardized EPA Protocol for Characterizing Indoor Air in Large Office Buildings*" (USEPA, 1994; USEPA, 2001). Data collected included volatile organic compounds, particulates, radon, microbiological contaminants (i.e., surface and airborne bacteria and fungi) sound, light, carbon monoxide, carbon dioxide, temperature and relative humidity, building characteristics, occupant symptoms and IAQ perceptions (Burton(a) et al., 2000; Burton(b) et al., 2000; USEPA, 2001; Womble et al., 1995). Parameters of building characteristics included occupancy, geographic location, ventilation, construction, smoking policy, water damage (past or present), fire damage, cleaning practices, pest control, and renovations (Burton(a) et al., 2000).

The 10 climatic regions in the BASE study covered the entire continental United States. To ensure that the ten various climates would be represented by the buildings selection, a stratified random sampling method was employed. Within the regions, cities having populations of ≥100,000 were randomly selected using a random numbers generator. Within the randomly selected cities, owners and tenants of buildings were identified and contacted for determination of building eligibility and for permission to include the building in the study. Next, buildings were screened to determine whether they had been publicized as "sick" or "problem." If not, a preliminary visit was scheduled to determine if at least one sampling space had >50 occupants and was being serviced by no more than two air handling units. Generally, three to five cities were studied in either a winter or summer testing period, with up to three buildings selected in each selected city (USEPA, 2001). After buildings meeting study criteria were identified and permission secured, buildings in each climatic region were randomly selected for inclusion in the study. The detailed randomization process is described elsewhere (USEPA, 2001).

BASE Study General Fungal Categories

The BASE Study grouped fungi into four general categories including 1) leaf-surface (phylloplane) fungi including *Alternaria*., *Cladosporium,* and *Epicoccum*; 2) soil fungi including *Aspergillus* and *Penicillium*; 3) water-requiring (hydrophilic) fungi including *Aspergillus fumigatus*, *Botrytis*, *Fusarium*, *Stachybotrys*, Yeast, *Sporobolomyces*, *Ulocladium*, and Zygomycetes; and 4) potentially toxigenic fungi including *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus versicolor*, *Fusarium*, and *Stachybotrys* (Macher et al, 2001).

Indoor and Outdoor Fungal Concentrations

The top seven most frequently identified outdoor airborne culturable fungal groups in the 789 BASE samples included *Cladosporium,* an unidentified grouping, *Penicillium, Aspergillus, Alternaria*, Yeast, and *Aureobasidium*, respectively. In comparison, *Cladosporium*, unidentified grouping, *Penicillium, Yeast, Aspergillus, Alternaria, and Aureobasidium*, respectively, were the most frequently identified groupings in the 1,767 indoor air samples (Macher et al., 2001).

In a BASE Study analysis of fungal groups identified in indoor and outdoor samples, (1% or more of the culturable samples), a higher frequency of the individual fungal groups were reported for outdoor samples as compared to indoor samples with the exception of *Botryosporium*, *Exobasidium*-like, *Rhinocladiella*-like, *Sporobolomyces*, *Thysanophora*, *Tritirachium,* and *Wardomyces* spp. (Macher et al., 2001). These seven fungal groups were not identified in more that 2% of the culturable samples. In addition, *Exobasidium*-like, *Thysanophora*, *Rhinocladiella*-like, *Botryosporium*, and *Wardomyces*, were only isolated from indoor air samples (Macher et al., 2001).

A cross-sectional study by Gaskin et al. (2012), and published later by Taylor et al., 2014, examined the occurrence of fungi in 19 “non-problem” office buildings in the 200 mile area

surrounding Adelaide, South Australia. The researchers reported that *Penicillium, Aspergillus*, *Cladosporium* and *Alternaria* were also the four most commonly isolated fungi in both the indoor and outdoor environments, as well as in summer and winter. However, their rank order was slightly different then the rank order observed in the BASE study. The temperatures in Adelaide range from an average maximum temperature of 84.2°F in the summer to an average low of 59°F in the winter. The average precipitation is approximately 21 inches per year. The Gaskin et al. (2012) study is one of the few studies, other than the BASE Study, to examine the occurrence of fungi in and outside of "non-problem" office buildings in various seasons. The findings highlight the high prevalence and widespread geographic occurrence of these four molds.

For office buildings without a history of water damage, outdoor air is generally the primary source of fungi indoors influencing both the diversity and concentration of fungi (Li and Kendrick, 1996; Shelton et al., 2002; NAS, 2017). Outdoor fungal exposure affected by seasonal variation may translate to longer-term trends as manifested by climate change, particularly with respect to temperature and humidity. This indoor-outdoor correlation will be a potential factor affecting human health if modifications to the HV/AC and building envelope in existing buildings do not keep pace with climate change and as the built environment continues to grow.

Seasonal Variation of Fungi

Frankel, et al (2012) examined indoor microbial exposures and their relationship to temperature, relative humidity, and air exchange rate, and reported that significant seasonal variation was detected (i.e., variation peaked during the summer and declined during the winter) for all indoor microbial exposures. These results expand on findings from numerous studies that

have demonstrated a clear relationship between indoor and outdoor air and respective exposures (Chao(a) et al., 2012; Frankel et al., 2012; Prussin and Marr, 2015; Lee et al., 2006, Macher et al., 2001) with one group of researchers reporting that outdoor air was a major driver of the indoor air microbiome (Prussin and Marr, 2015). BASE Study researchers reported that in most cases statistically significant pair-wise comparisons in the frequencies of identification of the four fungal categories were found between the samples collected indoors and outdoors in the summer and the winter. Overall a higher prevalence of the four fungal categories during the summer for both indoor and outdoor samples were noted with the exception of a non-significant finding for water-requiring fungi samples collected outdoors (Macher et al., 2001).

Fungi and Adverse Health Effects

Fungi perform essential roles in terrestrial ecosystems, decomposing dead organic matter and providing nutrient cycling and exchange. Their ubiquitous presence often places them in shared environments with humans, in some instances leading to various adverse health effects. Fungi detected in the indoor air of office buildings have been assessed for factors affecting health and comfort reported by building occupants (Chao(b) et al., 2003; Chapman et al., 2003; Fung and Hughson, 2003; Hodgson et al., 1998; Menzies et al., 1998; Menzies and Bourbeau, 1997). Additionally, the presence of water or high moisture levels within public and commercial building confines has been associated with contributing to the growth of mold and subsequent adverse effects on occupant health and comfort (Bardana Jr., 2003; Hardin et al., 2003; Mendell(a) et al., 2005; Mendell(b) et al., 2007). Potential health effects of exposure to fungi include infections, asthma, allergic rhinitis, hypersensitivity pneumonitis, allergic bronchopulmonary aspergillosis (ABPA), allergic fungal rhinosinusitis, and fungus balls (i.e., fungal mycelia) (Bush, 2018).

Study Rationale

Exposures to molds may produce a range of non-specific health symptoms, such as nasal, eye, and mucous membrane irritation, lethargy, headache, mental or cognitive distraction, dry skin, and asthma or respiratory distress. More severe and specific symptoms such as hemorrhage, infection, and allergy may also be manifested. The ubiquitous presence of fungi in our environment places the general population at risk for exposure, and in particular, among occupants in work spaces where fungi may be present in higher concentrations.

Fungal growth is influenced by temperature and moisture. As has been reported, the diversity and concentration of fungi found inside buildings without a history of water damage is influenced by the outdoor microbiome (Li and Kendrick, 1996; Shelton et al., 2002; NAS, 2017). The effects of climate change, particularly with respect changes in temperature and humidity, may influence the fungal microbiome through the diversity of the fungal species detected, or in their concentrations. Chapters 2 and 3 investigate the baseline concentrations and diversity for airborne fungi found in non-sick buildings. Chapter 4 expands upon these findings to examine whether there is a correlation between symptoms among the building occupants and the airborne fungal concentrations.

SPECIFIC AIMS AND HYPOTHESES

Specific Aim 1.1 - Examine the ratios between the concentrations of indoor culturable airborne office fungal concentrations and outdoor culturable airborne fungal concentrations.

Hypothesis 1.1: Concentrations of culturable airborne fungi in outdoor air will be higher than concentrations of the same species found in indoor air.

Hypothesis 1.2: The indoor-outdoor ratios of fungal species known to contribute to indoor levels will be less pronounced in regions with higher temperature and humidity.

For analyses, the various genera will be reported separately and summed for the total concentration of culturable molds (CFU/m^3). Concentration estimates for each building will be calculated by aggregating indoor measures into one averaged concentration, resulting in one indoor and one outdoor concentration. Indoor-outdoor ratios will be calculated using each building-wide average indoor concentration versus the corresponding outdoor concentrations. Regression analysis will be conducted on the aggregated concentrations of culturable airborne fungi to evaluate overall comparison between indoor and outdoor environments by region.

Specific Aim 1.2 - Examine the impact of seasonality (i.e., winter, summer) on the concentrations culturable airborne office fungal concentrations as well as outdoor culturable airborne fungal concentrations.

Hypothesis: Within study buildings and surroundings, indoor air and outdoor air in summer will have higher reported concentrations of culturable fungi as compared to indoor and outdoor air in winter.

Specific Aim 1.3 - Examine the impact of regional climatic factors (i.e., temperature and humidity) on the concentrations of culturable airborne office fungal concentrations as well as outdoor culturable airborne fungal concentrations.

Hypothesis: Indoor and outdoor air in climatic zones with higher temperatures and relative humidity will have higher reported concentrations of fungi as

compared to indoor and outdoor air of buildings in climate zones with lower temperature and humidity.

Climate regions will be stratified by temperature, humidity, and both, for both summer and winter. Linear and quadratic trend analyses will be performed for each of the measures of interest by climatic region and season. Whereas linear trend analyses will be employed to assess growth trends on the y axis (i.e., greater or lesser), quadratic trend analysis will enable assessment of *peak* growth, if any, given that fungi will have optimal growth temperatures with decreasing rates of growth at either lower or higher temperatures away from optimal. The analyses will better enable assessment of the potential effects of climate on detected presence of mold.

Specific Aim 2 - Examine the impact of regional climatic factors (i.e., temperature and humidity) on the diversity of airborne office fungi as well as outdoor fungal concentrations.

Hypothesis 2.1: Within climatic zones having higher humidity and temperature, diversity of detected fungi will be greater in both indoor and outdoor air as compared to diversity detected in climatic zones with lower humidity and temperature.

Hypothesis 2.2: The prevalence of commonly occurring fungal species detected outdoors will be the same as the prevalence of the species detected indoors.

A linear mixed model regression will be used to examine the diversity of airborne office and outdoor air. The effects will be: (1 climatic region, (2 airborne (Fungal air; indoor)/ outdoor airborne samples collected at the study building sites as described in the BASE Study protocol, and (3 diversity type. The relative differences will then be examined, with emphasis on

diversity. Measures will be characterized by major genus detected (> 5%) by climatic region, or in the case of species identification, by species. Data will be presented for each of the ten climate regions. Fungal diversity will be assessed using well-established indices of (1 species variety (number of groups observed) and (2 similarity of the abundance of the different fungal groups (Shannon evenness index) (Simpson, 1949; Shannon and Weaver, 1969). Similarity between fungal groups identified indoors and outdoors, and in summer and winter will be assessed using the Morisita-Horn similarity index (Horn, 1966; Wolda, 1981).

Specific Aim 3 - Examine the overall association between fungal concentrations detected in and outside the office buildings with self-reported respiratory and neurological health effects among building occupants.

Hypothesis: There is an association between fungal concentration and self-reported respiratory and neurological symptoms in the workplace.

For Aim 3, a cross sectional study design was performed to examine self-reported health effects of BASE Study building occupants who filled out health questionnaires based on a given time period of 4 weeks (n= 4,326). The study examined (1 the association between fungal concentrations with self-reported respiratory health effects among building occupants, (2 the association between fungal concentrations with self-reported neurological health effects among building occupants, and (3 the association between fungal concentrations and history of a chronic respiratory condition (i.e., asthma, hayfever, dust allergy, mold allergy, or chemical sensitivity). For the analytic plan, proportions and 95% confidence intervals of symptoms for respondents reporting worse respiratory and/or neurological symptoms in the office building (i.e., better at home) will be calculated. Logistic regression was performed and fitted using the general effects method to account for the clustering effects of questionnaire respondents occupying the same

buildings under study. Analyses to check for interaction effects of season and respiratory or neurological condition was then performed and included further analysis of interaction effects of years worked in building (> 5 years, ≤ 5 years).

OVERALL STUDY GOALS

The overall goal of this dissertation is to describe the regional occurrence of airborne indoor and outdoor fungal concentrations and to evaluate whether regional climatic differences impact fungal diversity, fungal concentrations, and building occupants' health. The nationwide scope of this study and the collection of regional data predating the heightened scientific awareness to global climate change presents a unique opportunity to establish baseline information on regional workplace fungal occurrences and concentrations that may be useful for predicting the impact of future climate change.

CHAPTER 2: AIRBORNE CULTURABLE FUNGI IN A CROSS-SECTIONAL STUDY OF LARGE OFFICE BUILDINGS IN THE UNITED STATES

INTRODUCTION

Prior to 1994, assessment of health effects in buildings was largely focused on "sick" buildings which, while important, did not present a comprehensive representation of conditions within the building stock in the United States. It became apparent that the need to identify and assess representative (i.e., typical) building conditions, as well as worker comfort and health, was necessary in order to develop informed policy with regard to potential need for future intervention or remediation. The Building Assessment and Survey Evaluation (BASE) Study was conducted by the EPA from 1994-1998 to provide insight into building stock and indoor air quality (IAQ)-related parameters and to fill a void in previously unknown baseline data among office buildings in the United States (USEPA 2001). The study was designed to establish baseline data for public and commercial buildings selected among a range of ten identified geographic climatic regions based on air conditioning and heating use and humidity (ASHRAE, 1989). In each of the 100 buildings included in the study, data were collected using sampling and assessment methodologies and a survey schedule defined in the EPA's protocol titled "*A Standardized EPA Protocol for Characterizing Indoor Air in Large Office Buildings*" (USEPA, 1994; USEPA, 2001). Data collected included volatile organic compounds, particulates, radon, microbiological contaminants including surface and airborne bacteria and fungi, sound, light, carbon monoxide, carbon dioxide, temperature and relative humidity, building characteristics, occupant symptoms and IAQ perceptions (Burton(a) et al., 2000; Burton(b) et al., 2000; USEPA, 2001; Womble et al., 1995). Parameters of building characteristics included occupancy,

geographic location, ventilation, construction, smoking policy, water damage (past or present), fire damage, cleaning practices, pest control, and renovations (Burton(a) et al., 2000).

To ensure that the ten various climates would be represented, a stratified random sampling method was employed for building selection. Within the regions, cities having populations of ≥100,000 were randomly selected using a random numbers generator. Within the randomly selected cities, owners and tenants of buildings were identified and contacted for determination of building eligibility and for permission to include the building in the study. Next, buildings were screened to determine whether they had been publicized as "sick" or "problem." If not, a preliminary visit was scheduled to determine if at least one sampling space had >50 occupants and was being serviced by no more than two air handling units. Generally, three to five cities were studied in either a winter or summer testing period, with up to three buildings selected in each selected city (USEPA, 2001).

The nationwide scope of this study and the collection of data pre-dating the heightened scientific awareness to global climate change presents a unique opportunity to establish baseline information on "non-sick" workplace fungal occurrence that may be useful for predicting the fungal-related impact of future climate change.

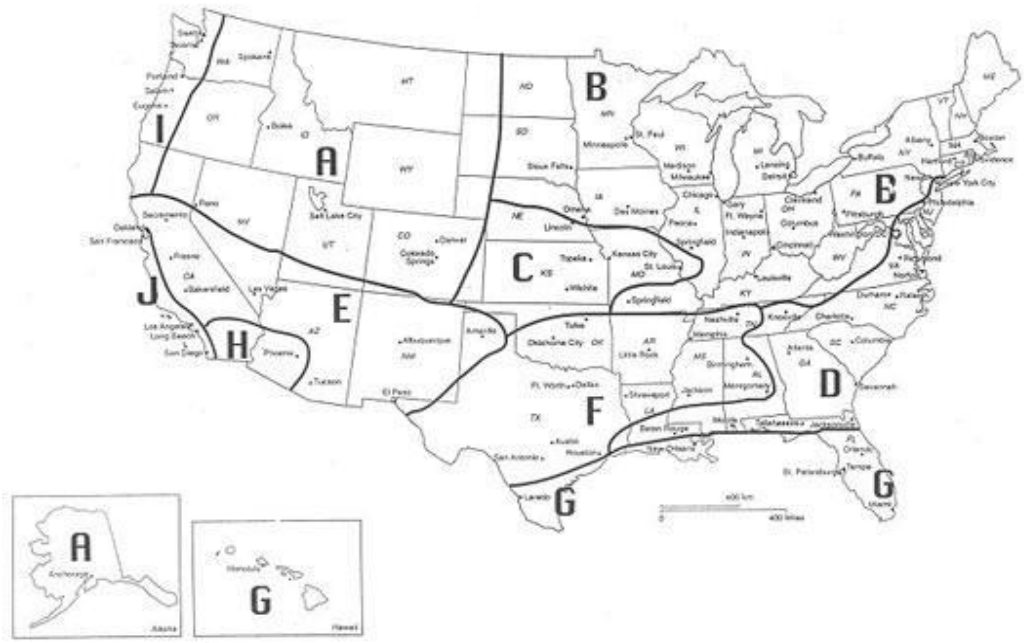
METHODS

Selection of Climatic Regions

The 10 climatic regions in the BASE study covered the continental United States and were based on the American Society of Heating, Refrigerating, and Air Conditioning Engineers (ASHRAE) specifications for building design, heating, and cooling requirements (ASHRAE, 1989; USEPA, 1994). Climate regions were assigned alphabetic designations (A-I) and were stratified by winter design temperature (Cool ≤ 10°F; Moderate 11°F -32°F; Hot > 32°F) and

summer design conditions under two dew point headings (Dew Point < 53°F: Temp. < 94°F or ≥94°F; Dew Point ≥ 53°F: Temp. < 94°F or ≥ 94°F) (USEPA, 1994; ASHRAE, 1989) (Figure 1).

Figure 1. Ten climate regions of the BASE study target cities.



Reference: American Society of Heating, Refrigerating and Air Conditioning Engineers, Inc., Chapter 26, Table 1 Climatic Conditions for the United States, in: "1989 ASHRAE Handbook Fundamentals."

For purposes of analysis, regions were ranked by increasing temperature and humidity. In instances where climate regions were equivalent in temperature, those with the higher humidity were assigned the higher rank.

Distribution of BASE Study Buildings by Climatic Region

The distribution of BASE Study buildings by climate region, state, and season is presented in Table 1 (ASHRAE, 1989). A representative number of buildings included within each climate zone was weighted by population living within each identified zone. For example, climate zone B encompassed a greater population than all other zones, and building numbers reflected this distribution. The distribution of buildings by region (A-J) and either summer (S) or

winter (W) testing period were: A(S) 3; A(W) 3; B(S) 11; B(W) 12; C(S) 2; C(W) 3; D(S) 9; D(W) 8; E(S) 4; E(W) 2; F(S) 8; F(W) 5; G(S) 4; G(W) 3; H(S) 2; H(W) 3; I(S) 3; I(W) 3; J(S) 6; J(W) 6.

Table 1. Building counts by climate region, state, and season.*

Region	State	Season	COUNT
A	CO	S	3
A	NV	W	3
B	IL	S	3
B	MA	W	3
B	MI	W	3
B	MN	W	3
B	NY	S	6
B	PA	S	2
B	SD	W	3
C	MO	S	2
C	NE	W	3
D	FL	W	3
D	GA	S	3
D	MD	S	3
D	NC	W	3
D	SC	W	2
D	TN	S	3
E	CA	S	1
E	CA	W	2
E	NM	S	3
F	AR	W	3
F	TN	S	3
F	TX	S	5
F	TX	W	2
G	FL	S	4
G	LA	W	3
H	AZ	S	2
H	AZ	W	3
I	OR	S	3
I	WA	W	3
J	CA	S	6
J	CA	W	6

*Reference: Summary and Analysis Report of the BASE Study Building Selection Process (USEPA 2001).

Forty-eight buildings across all climate zones were selected for inclusion in winter testing, and fifty-two distinct buildings (i.e., no overlap with buildings selected for winter study) were selected for summer testing, resulting in 100 total buildings in the study. Not all buildings tested in the one week test period in a given region or season were tested in the same calendar year, but were tested over the course of the 1994-98 study duration.

Descriptive Information for BASE Study Buildings

Building summary information for buildings selected for the BASE Study is presented in Table 2 (USEPA, 2001). Buildings were occupied 5-24 hours per day, with median daily occupancy of 10 hours/day. Ventilation was active from 0-120 hours/week, with a median ventilation time of 80 hours/week. Outdoor airstream CO_2 concentrations ranged from 327-536 ppm, with median concentrations of 375 ppm. Smoking was allowed in 25% of the buildings and prohibited in 75% of study buildings. Past water damage was recorded in 85% of all study buildings, with 43% showing evidence of current water damage of various degrees (i.e., water stains, presence of any water).

Table 2. Descriptive statistics of BASE study buildings (n=100).

Variable	Descriptive statistics
Hours per day building is occupied Median (IQR) Range	 10 (9-12) 5-24
Ventilation, hours per week Median (IQR) Range	 80 (60-120) 0-120
Average outdoor airstream CO2 concentration (ppm) Median (IQR) Range	n=96 375 (363-393) 327-536
Smoking allowed in building, count (%)	25 (25%)
Past water damage	85 (85%)
Current water damage	43 (43%)

Sampling of Fungi

For each of the 100 study buildings, sixteen air samples for culturable fungi were analyzed: (2 sampling durations: 2-/5-minutes) x (2 sampling periods: AM/PM) x (4 sets of samples: 3 indoor fixed sites + 1 outdoor site). Airborne samples were collected using Andersen N6 single-stage impactors using a flow rate of 28.3 ± 1.4 liters per minute. Fungal bioaerosols were impacted onto 100 x 15 mm petri plates containing 2% malt extract agar (MEA) supplemented with 20% dextrose. Samples were shipped overnight to a reference laboratory whereupon they were incubated at room temperature for seven days. Counts and morphological analyses were performed using light microscopy. Colony-forming units (CFUs) for each organism were enumerated in terms of number per unit volume of air sampled. For analyses, the various genera were reported separately and summed for the total concentration of culturable molds (CFU/m^3). Following BASE Study protocols where samples were below the limit of detection (LOD), a value of ½ LOD was used as an imputed value.

Statistical Analyses

Two-minute samples were used for the indoor analyses. Two-minute samples were used to avoid any chance of overloading the samples so that samples with high fungal counts were available to examine associations with self-reported health symptoms. A log average of the three 2-minute morning indoor samples produced one morning measure per building. Likewise, the log average of the three 2-minute afternoon indoor samples produced one afternoon measure per building. Both the morning and afternoon log average measures were then collapsed into one log average concentration measure per building. Data were analyzed at the building level. A linear mixed model was used for paired indoor and outdoor concentrations. Analysis was based on log scale. Distribution was log normal, and Shapiro-Wilkes test was used to test for normality. The

dependent variable was concentration of culturable airborne fungi. Univariate analysis included independent variables of season (Summer, Winter), location (indoor, outdoor), and climatic region (regions A-J). Mean concentrations were determined by back-transformation of the natural log mean, and confidence intervals were based on difference between the log scale repeated measures (e.g., log indoor fungal concentration minus log outdoor fungal concentration = e^x). All subsequent calculations were performed on log transformed values.

Climate regions were ranked by increasing temperature for both summer and winter. Linear and quadratic trend analyses were performed for each of the measures of interest by climatic region and season. Linear trend analyses was employed to assess growth trends on the y axis (i.e. greater or lesser), quadratic trend analysis was performed to assess potential *peak* growth, if any, to test optimal growth temperatures with decreasing rates of growth at either lower or higher temperatures away from optimal.

RESULTS

Concentrations of Culturable Airborne Fungi by Season

Concentration means for culturable airborne fungi (CFU/m^3) in indoor air, maximum indoor values, and outdoor concentrations are presented in Table 3. Mean average indoor air concentrations among study buildings ranged from 27.11 CFU/m^3 in winter to 46.02 CFU/m^3 in summer, and 35.69 CFU/m^3 overall. Standard deviations around the average indoor mean indicated wide variability, and maximum indoor measurements were also recorded to assess extent of variability during the study period. Means for maximum indoor values ranged from 45.79 CFU/m^3 in winter to 83.51 CFU/m^3 in summer. Mean outdoor values ranged from 189.24 CFU/m^3 in winter to 561.16 CFU/m^3 in summer with an overall mean of culturable fungi in outdoor air of 332.95 CFU/m^3. For all three measures (i.e., mean indoor, maximum indoor, and

mean outdoor), the summer measurements consistently ranked highest in concentrations of culturable airborne fungi detected, potentially indicating the influence of a temperature gradient on growth. Overall, the mean outdoor air fungal concentrations measured 7 to 10 times higher than the mean indoor air in winter and summer.

Table 3. Fungal concentrations: Mean indoor, Maximum indoor, Mean outdoor by season.

Variable (CFU/m^3)	Indoor			Maximum Indoor			Outdoor		
	n	Mean	SD	n	Mean	SD	n	Mean	SD
Fungal air (all)	100	35.69	36.80	100	62.61	65.62	100	332.95	390.89
Fungal air (summer)	52	46.02	43.90	52	83.51	80.01	52	561.16	511.21
Fungal air (winter)	48	27.11	28.47	48	45.79	48.53	48	189.24	222.16

Concentrations of Culturable Airborne Fungi in Summer and Winter by Region

Summer and winter mean concentrations of culturable airborne fungi in indoor and outdoor air from buildings were then stratified by climate region. Geometric means for airborne culturable fungi detected in summer among 52 study buildings and ten climate regions are shown in Table 4. Among the ten climate regions, Region B included the largest number of buildings (11), reflecting the largest population among the regions. Region D included 9 buildings, Region F, 8; Region J, 6; Regions E and G, 4 each; Regions A and I, 3 each; Regions C and H, 2 each. Mean indoor fungal concentrations, which ranged from 17.4 to 68.1 CFU/m^3, were analyzed for trend across all regions. Both linear and quadratic trend analyses were performed in order to assess whether fungal concentrations continuously trended higher across all zones with increasing temperatures, or whether airborne fungal presence was determined more by an optimal temperature with less presence detected at the lower and higher temperature/humidity zones. Both linear ($p = 0.549$) and quadratic ($p = 0.191$) trend for indoor measures did not reach significance. However, the linear trend for outdoor air culturable fungi across climate zones in

summer was statistically significant (p = 0.028), while quadratic trend was not (p = 0.145).

Confidence intervals were calculated to observe variability around the means.

Table 4. Fungal concentration (CFU/m^3) by climatic region—Summer.

Climate/Region *	n	Indoor			Outdoor		
		Mean**	95% CI		Mean**	95% CI	
1 (B)	11	50.0	28.5	87.5	1257.4	717.4	2203.7
2 (D)	9	45.5	24.5	84.6	654.2	351.8	1216.5
3 (I)	3	39.6	13.5	116.0	500.3	170.9	1465.1
4 (J)	6	43.2	20.2	92.4	388.3	181.7	830.1
5 (A)	3	17.4	5.9	51.0	238.1	81.3	697.4
6 €	2	53.7	14.4	200.3	459.5	123.2	1712.8
7 €	4	29.3	11.5	74.2	363.2	143.3	921.0
8 (F)	8	68.1	35.3	131.5	596.1	308.7	1150.9
9 (H)	2	44.6	12.0	166.1	161.2	43.2	600.9
10 (G)	4	64.8	25.5	164.2	436.6	172.2	1107.0
		Linear trend (p=0.549) Quadratic trend (p=0.191)			Linear trend (p=0.028) Quadratic trend (p=0.145)		

*Ranked by increasing temperature/humidity

**Geometric mean (95% CI) from back-transformation of natural log mean

Figure 2. Plot of summer mean (95% CI) fungal air concentration by climate region (CFU/m^3).

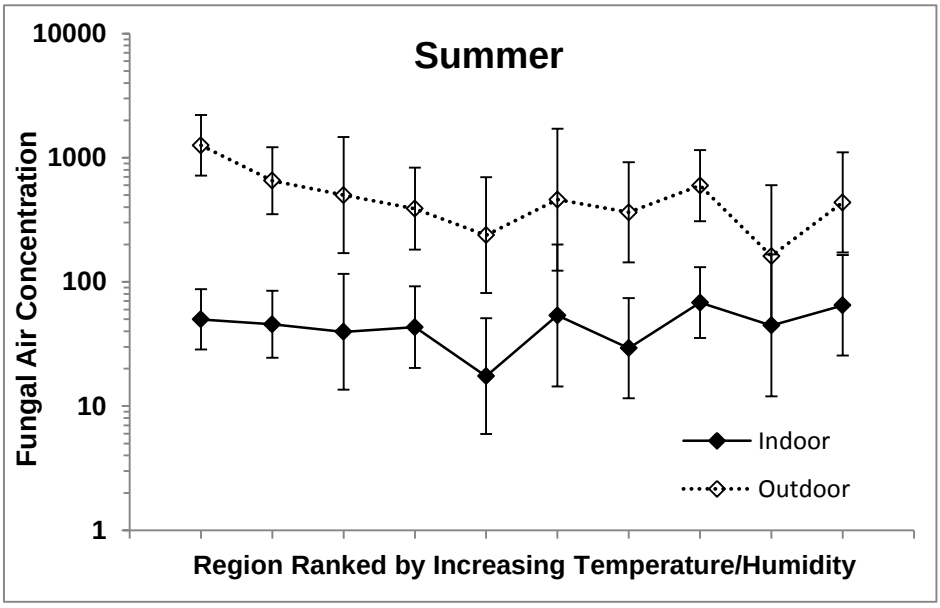


Figure 2 shows plotted means and 95% Cis for culturable airborne fungi in buildings during summer testing across the ten climatic regions for both indoor and outdoor measures. Outdoor means were higher than indoor means in buildings across all regions and show parallel trending.

Means for airborne culturable fungi detected in winter among 48 study buildings and ten climate regions are shown in Table 5. Among the ten climate regions, Region B included the largest number of buildings (12). Region D included 8 buildings, Region J, 6; Region F, 5; Regions A, C, G, H, and I, 3 each; Region E, 2. Mean indoor concentrations (range 12.7 to 107.4 CFU/m^3) were analyzed by both linear and quadratic trend analyses. Linear trend ($p = 0.372$) was not significant, whereas quadratic trend ($p = 0.061$) approached significance. Linear trend for outdoor air culturable fungi across climate zones in winter was highly statistically significant ($p = 0.004$), while the quadratic trend was not significant ($p = 0.205$).

Table 5. Fungal concentration (CFU/m^3) by climatic region—Winter.

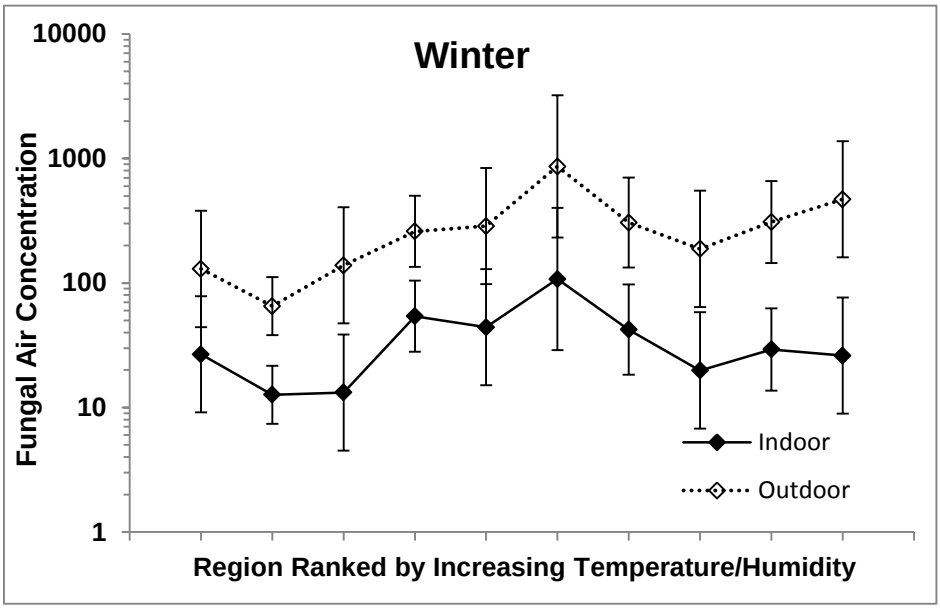
Climate/Region *	**n**	**Indoor**			**Outdoor**		
		Mean**	**95% CI**		**Mean****	**95% CI**	
1 (A)	3	26.7	9.1	78.2	129.7	44.3	379.7
2 (B)	12	12.7	7.4	21.7	65.2	38.1	111.5
3 €	3	13.2	4.5	38.7	138.6	47.3	405.9
4 (D)	8	54.0	28.0	104.3	260.1	134.7	502.2
5 (I)	3	44.1	15.1	129.2	286.5	97.9	839.0
6 €	2	107.4	28.8	400.5	862.7	231.4	3216.0
7 (F)	5	42.3	18.4	97.1	305.8	133.0	702.8
8 (G)	3	19.9	6.8	58.1	188.0	64.2	550.5
9 (J)	6	29.3	13.7	62.5	308.9	144.5	660.4
10 (H)	3	26.1	8.9	76.4	469.9	160.5	1376.0
		Linear trend (p=0.372) Quadratic trend (p=0.061)			Linear trend (p=0.004) Quadratic trend (p=0.205)		

*Ranked by increasing temperature

**Geometric mean (95% CI) from back-transformation of natural log mean

Figure 3 shows plotted means and 95% Cis for culturable airborne fungi during winter testing across the study climatic regions for both indoor and outdoor measures of study buildings. Similar to the summer observations, both indoor and outdoor detected fungal concentrations demonstrated parallel tracking.

Figure 3. Plot of Winter mean (95% CI) fungal air concentration by climatic region (CFU/m^3).



Ratio of Culturable Airborne Fungi in Summer and Winter by Region

The ratio of indoor total culturable airborne fungal concentration to outdoor total airborne culturable fungi in buildings by climate zone is summarized in Table 6. Ratios were calculated from back transforming natural log means, and analyzed for linear and quadratic trend.

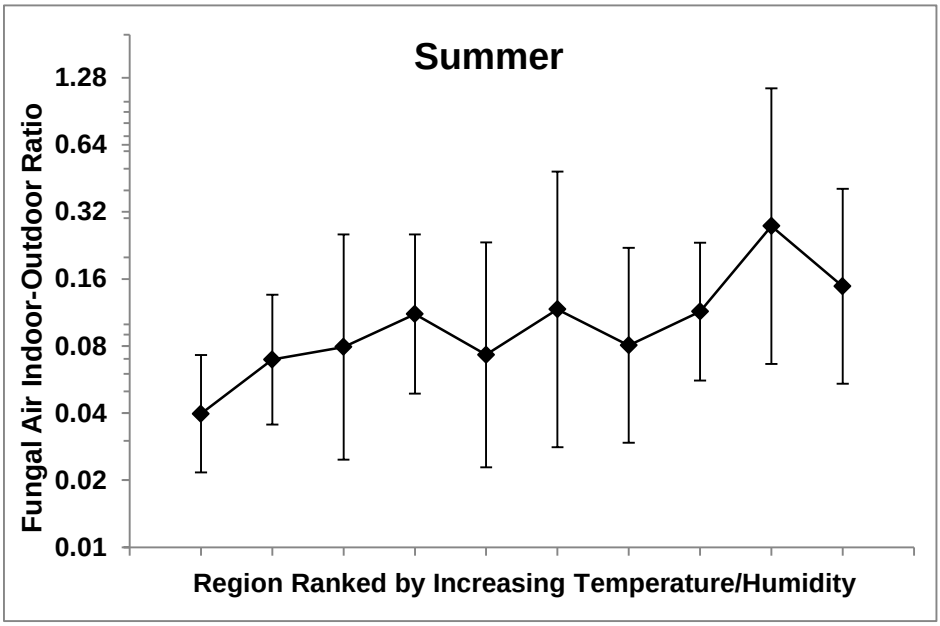
Table 6. Airborne fungal concentration indoor-outdoor ratio.

Summer				Winter			
Climate/Region*	**Mean****	**95% CI**		**Climate/Region***	**Mean****	**95% CI**	
1 (B)	0.040	0.022	0.073	1 (A)	0.206	0.064	0.659
2 (D)	0.070	0.036	0.136	2 (B)	0.194	0.109	0.348
3 (I)	0.079	0.025	0.253	3 €	0.095	0.030	0.305
4 (J)	0.111	0.049	0.253	4 (D)	0.208	0.102	0.423
5 (A)	0.073	0.023	0.234	5 (I)	0.154	0.048	0.493
6 €	0.117	0.028	0.486	6 €	0.125	0.030	0.518
7 €	0.081	0.029	0.221	7 (F)	0.138	0.056	0.340
8 (F)	0.114	0.056	0.233	8 (G)	0.106	0.033	0.338
9 (H)	0.276	0.067	1.149	9 (J)	0.095	0.042	0.215
10 (G)	0.148	0.054	0.406	10 (H)	0.056	0.017	0.178
Linear trend (p=0.010); Quadratic trend (p=0.889)				Linear trend (p=0.063); Quadratic trend (p=0.569)			

*Ranked by increasing temperature; Indoor significantly lower than outdoor (p<0.0001)
**Geometric mean (95% CI) from back-transformation of natural log mean

Means for summer ratios ranged from 0.040 (climate region B) to 0.276 (region H), and were plotted across climate zones ranked by increasing temperature (Figure 4).

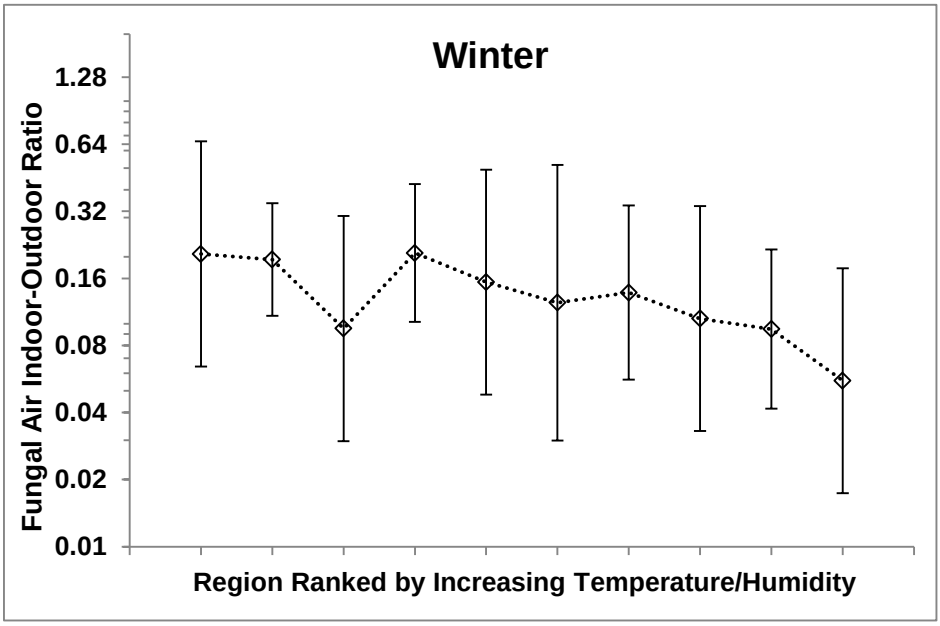
Figure 4. Plot of mean (95% CI) indoor-outdoor ratio of fungal air concentration by climatic region in Summer.



Linear trend for indoor-outdoor ratios (Figure 4) in buildings within all climatic zones ranked by increasing temperature was statistically significant (p = 0.010). Quadratic trend for peak growth across all climate regions was not significant (p = 0.889).

Means for winter ratios ranged from 0.056 (region H) to 0.208 (region D) and were plotted by climate zones ranked by increasing temperature (Figure 5). Linear trend was marginally significant (0.063), and quadratic trend did not demonstrate significance.

Figure 5. Plot of mean (95% CI) indoor-outdoor ratio of fungal air concentration by climatic region in Winter.



Occurrence of Airborne Fungal Groups

In addition to total culturable fungal concentrations, further refinement to a genus or species level was of interest in order to determine if indoor distribution reflected outdoor distribution. The ranking of total detected indoor airborne fungal groups is shown in Table 7. *Cladosporium* spp. was the most common fungal group identified to at least a genus level in indoor air, detected in 96 buildings and representing 12.63% of the total sample overall. *Penicillium* spp. was identified in 95 buildings and represented 12.5% of total detected sample.

Alternaria (47 buildings, 6.18% of sample) and *Aspergillus versicolor* (41 buildings, 5.39% of sample) were also detected over 5% of the total study sample. Although Non-Sporulating fungi and Yeasts comprised over 5% of the total sample (12.63% and 11.32%, respectively), the lack of an identified genus or species precluded meaningful discernment, other than to note their presence.

Table 7. Ranking of total detected indoor airborne fungal groups (n=760).

Fungal (air) – INDOOR	**COUNT**	**PERCENT**
CLADOSPORIUM	96	12.63
NON-SPORULATING	96	12.63
PENICILLIUM	95	12.50
YEAST	86	11.32
ALTERNARIA	47	6.18
ASPERGILLUS VERSICOLOR	41	5.39
AUREOBASIDIUM	34	4.47
UNKNOWN	33	4.34
ASPERGILLUS OTHER	32	4.21
ASPERGILLUS NIGER	27	3.55
COELOMYCETES	21	2.76
ASPERGILLUS FUMIGATUS	19	2.50
FUSARIUM	17	2.24
EPICOCCUM	13	1.71
PAECILOMYCES	12	1.58
CURVULARIA	11	1.45
ZYGOMYCETES	10	1.32
PITHOMYCES	8	1.05
ULOCLADIUM	7	0.92
ASPERGILLUS OCHRACEUS	6	0.79
BOTRYTIS	6	0.79
TRICHODERMA	6	0.79
DRECHSLERA	5	0.66
ASPERGILLUS GLAUCUS	4	0.53
SPOROBOLOMYCES	4	0.53
NIGROSPORA	3	0.39
WALLEMIA	3	0.39
ACREMONIUM	2	0.26
ASPERGILLUS FLAVUS	2	0.26
MONILIA	2	0.26
THYSANOPHORA	2	0.26

Table 7 continued		
TRITIRACHIUM	2	0.26
ARTHRINIUM	1	0.13
BOTRYOSPORIUM	1	0.13
EXOBASIDIUM-LIKE	1	0.13
NODULISPORIUM	1	0.13
OEDOCEPHALUM	1	0.13
RHINOCLADIELLA-LIKE	1	0.13
VERTICILLIUM	1	0.13
ZYGOSPORIUM	1	0.13

The ranking of total detected outdoor airborne fungal groups is shown in Table 8. *Cladosporium* spp. was detected in outdoor air at all study building sites (n= 100), and represented 11.2% of the total sample. *Penicillium* spp. was detected in outdoor air at 91 of the study buildings (10.19% of sample). *Alternaria* (65 buildings, 7.28% of sample) and *Aspergillus niger* (51 buildings, 5.71% of sample) were also detected at over 5% of the total sample. Non-sporulating fungi, Yeast, and Unknown were each detected at over 5% of the sample (11.09%, 8.73%, 5.26%, respectively) but were not identified to a genus or species level.

Cladosporium, *Penicillium*, and *Alternaria* each appeared in the same general order among indoor and outdoor culturable fungal groups identified to at least the genus level. However, differences were also revealed. Although detected at < 5% of the sample, several important species were notably different between indoor and outdoor airborne fungal groups detected. *A. versicolor* was detected in outdoor air samples at 23 buildings, but was found in indoor air in 41 buildings. *A. niger* was detected in outdoor air samples at 51 buildings and was found in indoor air in 27 buildings. Zygomycetes were notably divergent in the opposite direction, with more samples being detected in outdoor (31 buildings) than in indoor samples (10 buildings).

Table 8. Ranking of total detected outdoor airborne fungal groups (n=893).

Fungal (air) – OUTDOOR	COUNT	PERCENT
CLADOSPORIUM	100	11.20
NON-SPORULATING	99	11.09
PENICILLIUM	91	10.19
YEAST	73	8.17
ALTERNARIA	65	7.28
ASPERGILLUS NIGER	51	5.71
UNKNOWN	47	5.26
AUREOBASIDIUM	44	4.93
ASPERGILLUS OTHER	38	4.26
ZYGOMYCETES	31	3.47
EPICOCCUM	26	2.91
BOTRYTIS	25	2.80
ASPERGILLUS VERSICOLOR	23	2.58
FUSARIUM	23	2.58
ASPERGILLUS FUMIGATUS	19	2.13
COELOMYCETES	18	2.02
CURVULARIA	17	1.90
TRICHODERMA	15	1.68
DRECHSLERA	12	1.34
PITHOMYCES	12	1.34
PAECILOMYCES	10	1.12
ULOCLADIUM	10	1.12
ASPERGILLUS GLAUCUS	9	1.01
ASPERGILLUS FLAVUS	7	0.78
NIGROSPORA	5	0.56
ACREMONIUM	4	0.45
BEAUVERIA	2	0.22
MONILIA	2	0.22
TRITIRACHIUM	2	0.22
ARTHRINIUM	1	0.11
ARTHROSPORES	1	0.11
CUNNINGHAMELLA	1	0.11
NODULISPORIUM	1	0.11
OEDOCEPHALUM	1	0.11
OSTRACHODERMA	1	0.11
PESTALOTIA	1	0.11
SCOPULARIOPSIS	1	0.11
SPOROBOLOMYCES	1	0.11

Table 8 continued		
STACHYBOTRYS	1	0.11
VERTICILLIUM	1	0.11
WALLEMIA	1	0.11
ZYGOSPORIUM	1	0.11

DISCUSSION

The BASE Study provides data on airborne culturable fungi from 100 random-selected office buildings in ten geographic climatic regions across the continental United States. The cross-sectional, standardized, nationwide aspects of the BASE Study allows for examination of between-region and between-season variations of culturable airborne fungi from study buildings and exterior environments. Results from this study can be used as a baseline source of reference for investigation monitoring potential changes in fungal microbiomes in buildings among various climatic conditions as environmental conditions evolve. The essential environmental requirements for fungal growth—moisture, temperature, substrate—are inextricably linked to climate and often located within niches created and occupied by human populations within a built environment. Any change in essential growth requirements as a result in evolution of interior microclimates or the exterior macroclimate has the potential to affect the interaction with human populations and subsequent effects on health.

The data presented here may provide a reference to policy makers and health professionals interested in monitoring workplace IAQ, and provide baseline information for detecting temporal evolution of growing conditions or presence of airborne fungi in workplace building stock. At the initiation of the BASE study in 1994, large office buildings of the type included in the study represented 11% of office building stock in the U.S. and approximately 73% of all office workers (Brightman, 2005). This represented a solid basis from which to assess a sampling of non-sick, typical buildings in order to determine baseline characteristics and to

survey worker's health. Among the strengths of the BASE study was its comprehensive data collection in study buildings, its randomized building selection process, and nationwide extent across ten identified climatic regions.

Much was learned from the initial study, including an understanding of limitations encountered. In the BASE, only large office buildings were included, and different buildings were monitored within the common climate zones between summer and winter. However, the nationwide scope of the study helped identify "typical" conditions in these buildings across ten distinct climatic regions. Expression of certain fungal characteristics (production of toxins, spores, etc.) may be influenced by growing conditions and the substrates available, making detection of a particular species a non-uniform variable whose presence could affect health differently across multiple environments. Further, ascribing effects from one fungus when multiple fungi are present remains a substantial challenge.

The identification of culturable airborne fungi is dependent on researchers' ability to grow and detect them. In recognition of this limitation, in 1997—halfway through the main BASE study sampling—USEPA added airborne fungal spore sampling to aid in detection of fungal presence undetected by culture. The sampling was limited to 44 of the 100 BASE buildings in six climatic zones and is presented elsewhere (McIntosh, et al., 2014). Our current study focused on culturable fungi since a uniform testing regimen included data for all 100 study buildings and across all climatic regions, allowing us to examine potential effects across all climate regions.

The cross-sectional nature of the study provides a snap-shot within a set time, but use of validated methods and a randomized building selection provides representative measurements that may be extrapolated. Reaching valid conclusions from short-term data depends on

accumulated knowledge of the variability of airborne fungi over time (Burge, et al., 1997). However, care should be taken to recognize long-term trends beyond the shorter-term bounds of variability. The BASE Study and presentation of data from the present analysis, offers insight into a time prior to the present-day concerns about changes in climate, providing baseline information from which to visualize long-term temporal trends across various climatic conditions.

CHAPTER 3: DIVERSITY AND SIMILARITY OF INDOOR AND OUTDOOR FUNGI IN A NATIONAL SAMPLE OF OFFICE BUILDINGS

INTRODUCTION

Airborne fungi in indoor environments have been investigated as causes of adverse health effects (Bush and Portnoy, 2001; Chao et al., 2003; Fung and Hughson, 2003; Hardin et al., 2003; Harrison et al., 1992; Mendell et al., 2007; Menzies and Bourbeau, 1997; Menzies et al., 1998; Osborne et al., 2015; Portnoy et al., 2005). Increasingly, evidence suggests that the fungal microbiome found in indoor environments has a strong relationship with outdoor microbiomes (Jara et al., 2017; Lee et al., 2006; NAS, 2017; Prussin and Marr, 2015; Tsai et al., 2007) and is further influenced by seasonal variation associated with temperature and humidity (Frankel et al., 2012; Shelton et al., 2002), geographic regions (Amend et al., 2010; Shelton et al., 2002), and climate (Vardoulakis et al., 2015). However, there has been little published data comparing indoor and outdoor fungal populations across climatic regions in the United States with respect to diversity and evenness.

Fungi comprise a kingdom of eukaryotic organisms encompassing well over 100,000 described species (de Hoog, et al., 2000). The BASE Study grouped fungi into four categories which included 1) leaf-surface (phylloplane) fungi including *Alternaria*., *Cladosporium*, and *Epicoccum*; 2) soil fungi including *Aspergillus* and *Penicillium*; 3) water-requiring (hydrophilic) fungi including *Aspergillus fumigatus*, *Botrytis*, *Fusarium*, *Stachybotrys*, Yeast, *Sporobolomyces*, *Ulocladium*, and Zygomycetes; and 4) potentially toxigenic fungi including *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus versicolor*, *Fusarium*, and *Stachybotrys* (Macher et al, 2001).

The top seven most frequently identified outdoor airborne culturable fungal groups in the 789 BASE samples included *Cladosporium*, an unidentified grouping, *Penicillium*, *Aspergillus*,

Alternaria, Yeast, and *Aureobasidium*, respectively. In comparison, the most frequently identified groupings in the 1,767 indoor air samples were *Cladosporium*, unidentified grouping, *Penicillium*, Yeast, *Aspergillus*, *Alternaria*, and *Aureobasidium*, respectively. (Macher et al., 2001).

In a BASE Study analysis of fungal groups identified in indoor and outdoor samples, (1% or more of the culturable samples), a higher frequency of the individual fungal groups were reported for outdoor samples as compared to indoor samples with the exception of *Botryosporium*, *Exobasidium*-like, *Rhinocladiella*-like, *Sporobolomyces*, *Thysanophora*, *Tritirachium*, and *Wardomyces* spp. (Macher et al., 2001). These seven fungal groups were not identified in more than 2% of the culturable samples. In addition, *Exobasidium*-like, *Thysanophora*, *Rhinocladiella*-like, *Botryosporium*, and *Wardomyces*, were only isolated from indoor air samples (Macher et al., 2001).

A cross-sectional study by Gaskin et al. (2012), which examined the occurrence of fungi in 19 "non-problem" office buildings in the 200 mile area surrounding Adelaide, South Australia, reported that *Penicillium*, *Aspergillus*, *Cladosporium* and *Alternaria* were also the four most commonly isolated fungi in both the indoor and outdoor environments, as well as in summer and winter. However, their rank order was slightly different then the rank order observed in the BASE study. The temperatures in Adelaide range from an average maximum temperature of 84.2°F in the summer to an average low of 59°F in the winter. The average precipitation is approximately 21 inches per year. The Gaskin et al. (2012) study is one of the few studies, other than the BASE Study, to examine the occurrence of fungi in and outside of "non-problem" office buildings in various seasons. The findings highlight the high prevalence and widespread geographic occurrence of these four molds.

For office buildings without a history of water damage, outdoor air has been shown to be the primary source of fungi indoors influencing both the diversity and concentration of fungi (Li and Kendrick, 1996; Shelton et al., 2002; NAS, 2017). Outdoor fungal exposure affected by seasonal variation may translate to longer-term trends as manifested by climate change, particularly with respect to temperature and humidity. This indoor-outdoor correlation will be a potential factor affecting human health if modifications to the HV/AC and building envelope in existing buildings do not keep pace with climate change and as the built environment continues to grow. Analysis of diversity across climatic regions may offer additional insight into differences in the fungal microbiome and aid in predicting effects due to changes in climate.

METHODS

Fungal Sampling

Two-minute samples were collected in both the morning and afternoon at three indoor locations in each study building. Two minute samples were used to avoid any chance of overloading the samples so that samples with high fungal counts were available to examine associations with self-reported health symptoms. In addition, samples were also taken in the morning and afternoon sampling rounds outdoors near the air intake source of the air handling unit serving the indoor test space. Sampling methodology was the same for both indoor and outdoor collections. Airborne samples were collected using Andersen N6 single-stage impactors using a flow rate of 28.3 ± 1.4 liters per minute. Fungal bioaerosols were impacted onto 100 x 15 mm petri plates containing 2% malt extract agar (MEA) supplemented with 20% dextrose. Samples were shipped overnight to a reference laboratory whereupon they were incubated at room temperature for seven days. Counts and morphological analyses were performed using light microscopy. Colony-forming units (CFUs) for each organism were enumerated in terms of

number per unit volume of air sampled, allowing two-minute samples to be analyzed in aggregate (volume-weighted average) for the separate indoor and outdoor sampling sets.

Assessment of Fungi Evenness (E) and Similarity (S)

Fungal diversity was assessed across regional groups using three established indices: (i) species variety (S[1]) (Wolda, 1981), (ii) the degree to which the fungal groups occurred in equal proportion (Shannon diversity index, H')[2], (Shannon and Weaver, 1969) and (iii) evenness (Shannon evenness index, E)[3], (Simpson, 1949; Shannon and Weaver, 1969). Similarity between fungal groups detected indoors or outdoors and in summer or winter were then observed using the Morisita-Horn similarity index (C_{MH})[4], (Horn, 1966; Wolda, 1981).

Species variety was examined by observing the detected fungal groups and their frequencies among the indoor and outdoor collections. The mean number of *different* fungal groups detected at each study building was also calculated for observed indoor and outdoor bioaerosols in each of the climate regions and for both summer and winter periods; distinct buildings were tested in each season. Evenness of fungal groups was examined by first ranking regions by temperature/humidity and then further combining the regions into three larger groups while maintaining rank order. The larger groupings were formed in order to account for and to equalize the wide disparity in numbers of buildings tested by region. A minimum of three

[1] S = the number of groups observed
$p_i = n_i/N$
n_i = total number of samples in which fungal group was observed for the ith fungal group, where i = 1 to S
N = total observations for all fungal groups; $N = \Sigma(n_i)$.
[2] $H' = -\Sigma p_i \ln p_i$
[3] $E = H'/\ln S$
[4] $C_{MH} = 2\Sigma(an_i bn_i)/[da + db)(aN\ bN)]$ where
an_i = total observations for the ith fungal group in sample series A,
bn_i = total observations for the ith fungal group in sample series B,
aN = total observations in series A,
bN = total observations in series B,
$da = \Sigma an_i^2/aN^2$
db = $\Sigma bn_i^2/bN^2$

climate regions each were grouped representing the highest and lowest temperature/humidity designations in each of the seasons; a third group included the remaining four climate regions. Evenness was calculated in order to observe how close in number each species/group was between the three region groups.

RESULTS

Fungal Groups Detected

In the 100 buildings studied, 40 different fungal groups were identified indoors and 42 different fungal groups were identified outdoors. The most common groups identified indoors and outdoors and among the three combined climate groups were *Aspergillus* spp. (combined species), *Cladosporium* spp., *Penicillium* spp., Non-sporulating fungi, Yeasts, and *Alternaria*. *Botrysosporium* spp., *Thysanophora*, *Exobasidium*-like and *Rhinocladiella*-like groups were only found indoors and at low concentration and frequency. Among the Aspergilli identified indoors to the species level, *A. versicolor* was the most frequently detected (41 samples), followed by *A. niger* (27 samples) and *A. fumigatus* (19 samples). Among outdoor samples, 41 different fungal groups were identified. *Cladosporium* spp and Non-sporulating fungi were the most common, and found in the outside test area in 96 samples. *Penicillium* spp. (95 samples) and *Alternaria* (47 samples) were also found with higher frequency in the outdoor environment.

Table 9. Number of species groups in fungal air (mean per building)—Summer.

Climate/Region *	n	Indoor			Outdoor		
		Mean	95% CI		Mean	95% CI	
1 (B)	11	7.5	6.1	9.0	9.6	8.2	11.1
2 (D)	9	8.9	7.3	10.5	9.0	7.4	10.6
3 (I)	3	9.7	7.0	12.4	11.7	9.0	14.4
4 (J)	6	7.8	5.9	9.8	8.7	6.7	10.6
5 (A)	3	6.7	4.0	9.4	9.0	6.3	11.7
6 (C)	2	7.5	4.2	10.8	13.0	9.7	16.3
7 (E)	4	8.8	6.4	11.1	10.5	8.1	12.9
8 (F)	8	9.8	8.1	11.4	10.0	8.3	11.7
9 (H)	2	8.5	5.2	11.8	10.0	6.7	13.3
10 (G)	4	9.0	6.6	11.4	9.5	7.1	11.9
		Linear trend (p=0.483) Quadratic trend (p=0.544)			Linear trend (p=0.734) Quadratic trend (p=0.290)		

*Ranked by increasing temperature/humidity

Table 10. Number of species groups in fungal air (mean per building)—Winter.

Climate/Region*	n	Indoor			Outdoor		
		Mean	95% CI		Mean	95% CI	
1 (A)	3	5.3	2.6	8.0	7.3	4.6	10.0
2 (B)	12	5.3	4.0	6.7	5.9	4.6	7.3
3 (C)	3	6.0	3.3	8.7	10.0	7.3	12.7
4 (D)	8	8.0	6.3	9.7	7.3	5.6	8.9
5 (I)	3	5.7	3.0	8.4	6.0	3.3	8.7
6 (E)	2	10.0	6.7	13.3	11.5	8.2	14.8
7 (F)	5	7.6	5.5	9.7	9.8	7.7	11.9
8 (G)	3	6.3	3.6	9.0	9.0	6.3	11.7
9 (J)	6	7.7	5.7	9.6	9.8	7.9	11.8
10 (H)	3	6.0	3.3	8.7	9.7	7.0	12.4
		Linear trend (p=0.223) Quadratic trend (p=0.073)			Linear trend (p=0.012) Quadratic trend (p=0.667)		

*Ranked by increasing temperature

Table 9 and Table 10 display means and 95% confidence intervals for fungal groups observed by study building and by climate region for both summer and winter, respectively.

Results were plotted for each season in order to visualize comparisons between indoor and outdoor means and to visualize trends (Figure 6, summer; Figure 7, winter). Among buildings tested in the summer, those in Regions F and I exhibited the highest means for fungal

groups observed indoors (9.8, 95%CI (8.1- 11.4); 9.7, 95%CI (7.0- 12.4), respectively). Region A exhibited the lowest mean number of groups per building tested (6.7). The mean number of groups detected in outdoor air was highest in Region C (13.0) and Region I (11.7). The largest difference in numbers between indoor and outdoor fungal groups detected was Region C (7.5 vs 13.0 groups, respectively). The means presented in Table 9 were then plotted by climate region order (summer) for both indoor and outdoor measures (Figure 6). Both linear and quadratic tests for trends did not reach significance.

Figure 6. Plot of summer mean (95% CI) number of species groups per building (region summer order).

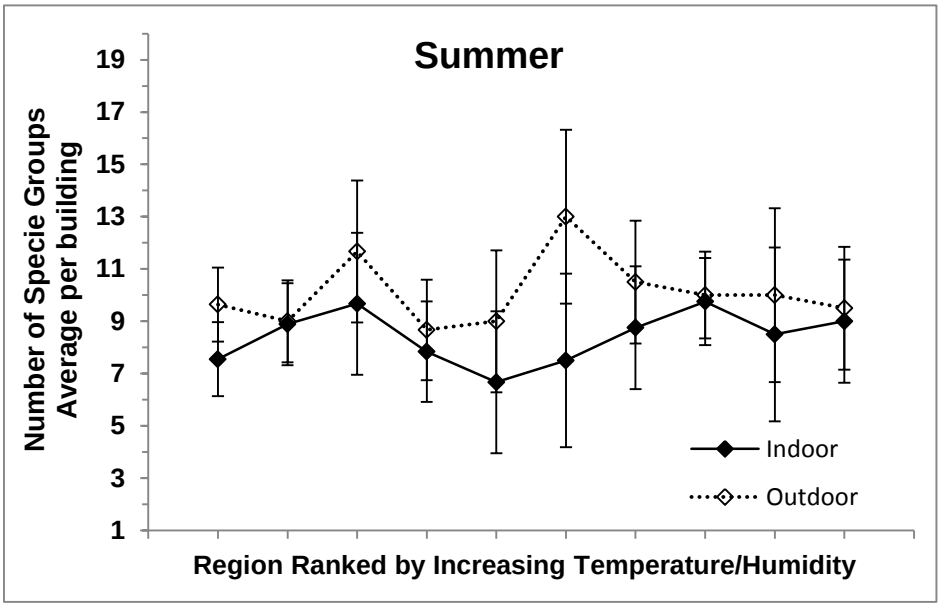
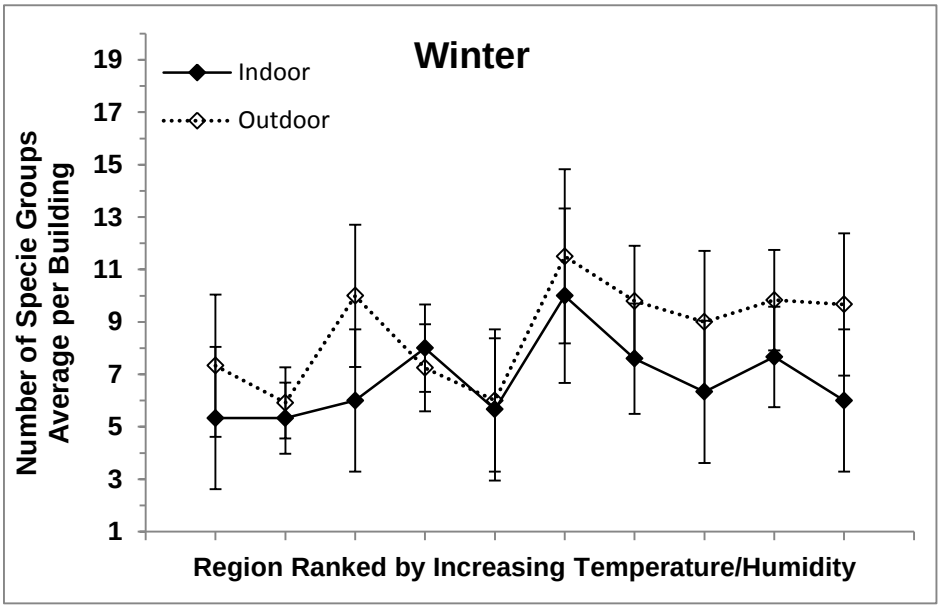


Figure 7. Plot of winter mean (95% CI) number of species groups per building (region winter order).



The means for numbers of culturable fungal groups comparing indoor and outdoor air by region, ranked by temperature and humidity in winter are shown in Table 10. The mean number of fungal groups detected outdoors generally was higher than detected indoor, per building. However, Region D exhibited a higher mean number of species detected indoor than outdoor (8.0 vs 7.3 groups, respectively). The highest mean species groups detected in study buildings both indoor and outdoor was observed in Region E (10.0 and 11.5 groups, respectively). The means presented in Table 10 were then plotted by climate region winter order for both indoor and outdoor measures. Linear trend was highly significant across ranked climate regions for number of species detected, indicating a temperature effect on numbers of species as temperatures became more moderate. In addition, quadratic trend for number of species detected in indoor air approached significance ($p = 0.073$).

Fungi Evenness

Evenness is a measure of biodiversity which quantifies how equal a community of organisms is numerically, and how close in numbers each species is in an environment. Species evenness ranges from zero to one, with zero signifying no evenness and one indicating complete evenness. In order to analyze fungal group evenness, the ten climate regions previously ranked by temperature/humidity and season were combined into three groups with roughly equivalent numbers of buildings in each group. Evenness among the climate groups is presented in Table 11. Evenness was consistent among species groups in indoor air in both summer (range 0.858 – 0.878) and winter (range 0.863 – 0.878). In outdoor air, evenness was high, but showed greater variation among species groups in both summer (range 0.875 – 0.910) and winter (range 0.853 – 0.904). The highest evenness among fungal groups was consistently seen in outdoor air in the climate group having the higher ranked temperatures in both summer and winter.

Table 11. Species evenness (E) among regional groups.

Region Groups	**Summer**	
	Indoor	**Outdoor**
1-3 (B, D, I)	0.876	0.875
4-7 (J, A, C, E)	0.858	0.895
8-10 (F, H, G)	0.878	0.91
	Winter	
	Indoor	**Outdoor**
1-3 (A, B, C)	0.863	0.853
4-7 (D, I, E, F)	0.863	0.888
8-10 (G, J, H)	0.878	0.904

Fungi Similarity

Species group similarity is a measure *between* communities—in this case the culturable fungi between climate groups. Similarity between the climate groups is presented in Table 12.

Table 12. Species similarity (CMH) among regional groups.

Region Groups	**Summer**	
	Indoor	**Outdoor**
(1-3)(4-7)	0.944	0.957
(1-3)(8-10)	0.925	0.935
(4-7)(8-10)	0.938	0.955
	Winter	
	Indoor	**Outdoor**
(1-3)(4-7)	0.929	0.917
(1-3)(8-10)	0.948	0.921
(4-7)(8-10)	0.939	0.951

Species groups among climate groups were similar across the BASE study (range 0.921 – 0.957). This finding was not unexpected, but particular focus was directed to any dis-similarity between the higher-temperature regions and the lower-temperature regions to detect differences in the fungal microbiome across a spectrum of temperature. Species groups were least similar among the low temperature versus high temperature climate groups in both indoor and outdoor air in the summer (Similarity index of 0.925 and 0.935 respectively) when observed in context with other comparisons.

DISCUSSION

Distribution of the observed fungal groups was dominated by a few groups which were present in higher concentrations, overshadowing potentially important groups found in lower concentrations but which could be contributors to occupant symptoms. Although cause-effect to

health is complicated by this overshadowing, large cross-sectional studies are particularly useful in establishing baseline diversity and evenness measures across multiple climates which can be used in conjunction with smaller longitudinal studies having more limited scope.

Fungal biodiversity in environments is considered highly diverse when a large number of detected groups generally are found to be equally abundant. This was not observed in the BASE study. Although C_{MH} agreement between the (i) fungal groups and (ii) occurrence of fungi detected indoors and outdoors was high among climate groups, the outdoor environment was consistently more diverse than indoors, and summer seasonal testing revealed higher richness than that detected in winter. The higher outdoor concentrations of culturable fungi lend support to the assumption that the outdoor environment is a major contributing source of the indoor fungal microbiome. Further, lower similarity of species groups between the most disparate climate groups in both indoor and outdoor air in the summer (Similarity index of 0.925 and 0.935 respectively) warrants further study with respect to evolving climate.

Studies have employed protocols similar to BASE, measuring indoor- outdoor culturable fungal concentrations on a smaller scale (Reynolds et al., 2001; Spicer and Gangloff, 2005). However, the comprehensive scope of BASE normative measures of culturable fungi in non-complaint (non-sick) office buildings will be useful in the development of health guidelines for indoor exposures, particularly when used with accumulated findings from smaller-scale cross-sectional studies and longitudinal studies. Understanding relationships between microbiome diversity and seasonal variation and climatic conditions, as well as indoor-outdoor ratios of fungal groups will further this process.

CHAPTER 4: MEASURES OF CULTURABLE AIRBORNE FUNGI AND SELF-REPORTED HEALTH EFFECTS

INTRODUCTION

Fungi detected in indoor environments have elicited increasing attention as causes of adverse health effects among humans (Bardana Jr., 2002; Bush and Portnoy, 2001; Chao et al., 2003; Epstein and Fan, 2001; Etzel et al., 1998; Fung and Hughson, 2003; Hardin et al., 2003; Harrison et al., 1992; Hodgson et al., 1998; IOM, 2011; Johanning et al., 1996; Koskinen et al., 1999; Mendell et al., 2007; Menzies and Bourbeau, 1997; Menzies et al., 1998; NAS, 2017; Shelton et al., 2002). Exposures to fungi and their by-products (i.e., toxins, VOCs) have been associated with health problems, including respiratory ailments and allergies, irritations of the skin and respiratory tract, toxic effects, and infections (Epstein and Fan, 2001; Etzel et al., 1998; Hardin et al., 2003; Hodgson et al., 1998; Johanning et al., 1996; Li and Kendrick, 1995; Mendell et al., 2011; Osborne et al., 2015; Portnoy, et al., 2005;).

Presently, there are no government standards that specify allowable concentrations of airborne fungi detected inside buildings. Data assimilated from fungal airborne samples,— both indoor and outdoor,— can be useful in evaluation and interpretation of the relationship between health hazards and adverse health effects, and can aid in further development of informed health policy despite a lack of set standards. Additionally, there has been scant published data comparing indoor and outdoor fungal species populations or whether those populations differ across climatic regions in the United States. Data forming a temporal reference baseline has been limited with respect to climate regions, although Sheldon et al., examined profiles of airborne fungi across geographic regions. Analysis across climatic regions may offer additional insight into climatic differences influencing the indoor workplace fungal microbiome and, more

broadly, serve as a baseline for predicting health effects due to warming trends in climate. Without comparative reference, the significance of qualitative and quantitative observations in future studies of airborne fungi and related health effects will be all the more challenging.

In buildings that are not water-damaged, fungi generally enter the building through leakage in the building envelope and through ventilation systems, but may also be carried indoors by occupants or introduced with new building materials (NAS, 2017). However, outdoor air has been observed to be the prevailing source of fungi found indoors (Chao et al., 2003; Frankel et al., 2012; Lee et al., 2006; Prussin and Mar, 2015). As a result, comparisons with outdoor air are important in the assessment of indoor fungal populations, particularly with respect to moisture conditions and temperature variation due to seasonal changes and their association with human health (NAS, 2017). At the time the BASE study was conducted, climate change had not yet reached its current level of interest and concern as noted in the Institute of Medicine's 2011 report, *Climate Change, the Indoor Environment, and Health* (IOM, 2011). Whereas certain species of fungi are known to thrive among specific climatic parameters (Chao et al., 2003), temperature and humidity may alter the indoor microbiome as climate changes, creating a cascade of evolving health effects due to these changes. As part of the BASE Study, health symptoms of building occupants located in 10 identified climate zones were recorded from answers to a questionnaire, which provided the EPA with a clearer understanding of existing normative IAQ data and occupant-experienced symptoms in the study office buildings located across the United States. Analysis of raw data from the study has resulted in numerous studies and publications. However, a clear need for further research and data exists with respect to health symptoms and the denominator of climate.

BASE Study data have been analyzed in many focused areas of interest, but in order to fill a knowledge gap, this approach will be to examine trends across the range of climatic conditions in the continental United States, and to examine baseline reported health effects across the ten American Society of Heating, Refrigerating and Air-Conditioning Engineers (ASHRAE, 1989) identified climatic zones in the United States to determine whether there are differences in self-reported health effects among building occupants across climate zones. The goal was to assess whether baseline observations of self-reported health effects differ as a result of seasonal or climatic differences and to provide a comparative foundation for data observed in future studies.

METHODS

Human Subjects Approval

Information and a project description was submitted to the University of Iowa Human Subjects Office/Institutional Review Board prior to initiation of project work. It was determined since the activity was limited to analysis of environmental data and since questionnaire data were not linked to identified individuals, human subjects approval was not required.

BASE Questionnaires and Variables of Interest

The U.S. EPA conducted an *Indoor Environmental Quality Survey* (US EPA, 1994) by distributing questionnaires among occupants of BASE Study buildings at the time the buildings were under observation. Normative and baseline data regarding indoor air quality in study buildings as well as occupant health and basic demographics were sought on a voluntary and confidential basis. The questionnaire focused on areas of interest: description of office work areas (cleanliness, lighting, etc.); health and well-being; environmental conditions; basic job

responsibilities and satisfaction. Demographic information (e.g., number of years worked in building, smoking status, perceived sensitivity to chemicals, age category, sex) was also collected. Self-reported chronic health information was obtained by prefacing with the question "Have you ever been told by a doctor that you have…" migraines; asthma; eczema; hay fever; allergy to dust; allergy to mold? The questionnaire also sought information about whether reported symptoms reduced one's ability to work or caused one to stay at home. Environmental conditions and comfort were sought for perceived conditions such as too hot or too cold, too dry or too humid, whether tobacco odors were present and whether noise levels were a concern. Education categories, satisfaction with one's job, and interaction with one's colleagues rounded out the collection of data.

Self-reported health effects of BASE Study building occupants who filled out health questionnaires (n= 4,326) were based on a given time period totaling 4 weeks immediately preceding administration of the questionnaire. Respiratory symptom variables included shortness of breath, chest tightness, cough, sinus congestion, sneezing, sore throat and wheezing. Neurological symptom variables were: difficulty concentrating, dizziness, fatigue, headache, irritability, nausea, and tired eyes. General symptoms of dry skin and dry eyes were also examined. Respondents reported frequency and severity of respiratory and neurological symptoms in response to the following questions:

During the last four weeks you were at work, how often have you experienced the following symptoms?

1. Not in the last 4 weeks
2. 1-3 days in the last 4 weeks
3. 1-3 days per week in the last 4 weeks

4. Every, or almost every workday

During the last 4 weeks you were at work, what happened to the symptom at times when you were away from work?

1. Got worse
2. Stayed same
3. Got better

The presence of a respiratory symptom was defined as Yes/No and coded "Yes" when the respondent reported the symptom *at least* 1-3 days per week in the last 4 weeks and reported the symptom "stayed same" or "got better" when away from work. The presence of a neurological symptom was defined as Yes/No and was coded "Yes" when the respondent reported the symptom *at least* 1-3 days per week in the last 4 weeks and reported the symptom "stayed same" or "got better" when away from work. The presence of a chronic respiratory condition was reported if the respondent responded "Yes" to having a history of at least one of the following conditions: asthma, hayfever, dust allergy, mold allergy, or chemical sensitivity). Variables and covariables are listed in Table 13.

Design and Data Analysis of Health Effects

The study examined (1- the association between airborne fungal concentrations with self-reported respiratory health effects among building occupants, and (2- the association between airborne fungal concentrations with self-reported neurological health effects among building occupants. Proportions and 95% confidence intervals of symptoms for respondents reporting worse respiratory and/or neurological symptoms in the office building (better at home) were calculated. Logistic regression was performed and fitted using the General Estimating Equations (GEE) method to account for the clustering effects of questionnaire respondents occupying the

same buildings under study, and was adjusted for covariates (season, respiratory condition [reported history of asthma, hayfever, dust allergy, mold allergy, chemical sensitivity], history of tobacco use, and building characteristics [smoking allowed in building, past or current water damage, hours building occupied, ventilation hours, CO_2 output]).

Three different models were fitted. All variables were selected a priori and were selected as biologically relevant. In the first, the dependent variable was the presence of a respiratory *symptom* (shortness of breath, chest tightness, cough, sinus congestion, sneezing, sore throat, wheezing). The independent variable was concentration of culturable airborne fungi—average indoor, maximum indoor, outdoor—each analyzed separately. Analyses to check for interaction effects of season or history of chronic respiratory condition and respiratory symptoms were performed. In the second model, the dependent variable was the presence of a neurological *symptom* (difficulty concentrating, dizziness, fatigue, headache, tension/irritability, nausea, tired/strained eyes). The independent variable was, again, concentration of culturable airborne fungi—average indoor, maximum indoor, outdoor. Analyses to check for interaction effects of season or history of chronic respiratory condition and respiratory symptoms were performed. In the third model, the dependent variable was the presence of a chronic respiratory *condition* (asthma, hayfever, dust allergy, mold allergy, chemical sensitivity). The independent variable in the third model was concentration of culturable airborne fungi—average indoor, maximum indoor, outdoor. Analyses to check for interaction effects of season or years worked in the building and respiratory symptoms were performed. It was important to adjust for those individuals reporting a known respiratory *condition* in order to distinguish from those with no condition, yet who were experiencing a respiratory *symptom*. Since the goal was to assess the effect of each one of those variables that was already known, model building to select variables

was not performed, and univariate analysis was not conducted. All variables described were included in the model and adjusted using the GEE method.

Table 13. Variables and Covariables

Variables of Interest	**Measure**	**Summary of Variables**
Airborne Culturable Fungi	CFU/m^3	Concentration
Respiratory Symptoms	Self-reported, experienced within 4 weeks of response (questionnaire)	Shortness of breath, chest tightness, cough, sinus congestion, sneezing, sore throat, wheezing
Neurological Symptoms	Self-reported, experienced within 4 weeks of response (questionnaire)	Difficulty concentrating, dizziness, fatigue, headache, irritability, nausea, tired eyes
Dry Skin	Self-reported (questionnaire)	Eczema, dry skin
Dry Eyes	Self-reported (questionnaire)	Dry eyes
Respiratory Condition (Covariate)	Self-reported (questionnaire) "have been diagnosed…"	Asthma, hay fever, dust allergy, mold allergy, chemical sensitivity
Season (Covariate)	Season when measures taken	Summer, Winter
Building Characteristics (Covariates)	Y/N Y/N Hours/week Hours/week ppm	Smoking allowed in building past or current water damage hours building occupied ventilation hours CO_2 output
Demographics (Covariates)	Male/Female Years Y/N	Sex years worked in building history of tobacco use

RESULTS

Clinical Conditions and Demographics of Building Occupants

Clinical conditions and demographics of study building occupants responding to the BASE questionnaire (n= 4326) are described in Table 14. Ages were grouped categorically. Among respondents, 16.2% were younger than 30 years of age; 28%, 30-39; 32.6%, 40-49; 18.7%, 50-59; 4%, ≥ 60. Among respondents, 34.1% were male and 65.9% were female. Years worked in a building ranged from 0.04 years to 42 years, with 1904 (44.5% of respondents) working in a study building ≥ 5 years. Clinical conditions reported included "any respiratory condition" (67.2%, sensitivity to chemicals in the air (49%), tobacco use (41.5%), dust allergy (31.9%), hay fever (28.8%, mold allergy (25.3%) migraine (21.5%), asthma (12.4%), eczema (8.6%).

Table 14. Clinical conditions and demographics of building occupants (n=4326).

Variable	n	Count (%)
Any respiratory condition	4253	2858 (67.2%)
Asthma	4032	499 (12.4%)
Hay fever	4073	1174 (28.8%)
Dust allergy	4158	1328 (31.9%)
Mold allergy	4093	1034 (25.3%)
Sensitivity to chemicals in the air	4276	2094 (49.0%)
Migraine	4099	880 (21.5%)
Eczema	3972	343 (8.6%)
Tobacco use (current/past)	4304	1788 (41.5%)
Sex (Male)	4295	1463 (34.1%)
Age	4294	
<30		697 (16.2%)
30-39		1223 (28.5%)
40-49		1399 (32.6%)
50-59		805 (18.7%)
≥60		170 (4.0%)
Years worked in building	4278	
(≥5 years)		1904 (44.5%)
Median (IQR)		4 (1-8)
Range		0.04-42

Home versus Office Symptoms

Questionnaire respondents were asked to report whether respiratory and neurological symptoms being experienced (if any) were either the *same* or better at home, or clearly better at home (worse at work) for the duration of the 4-week test period. The proportions and 95% confidence intervals for respondents reporting *any* symptom either in the summer or winter test period and for which the symptom was the same or better at home are presented in Table 15.

Table 15. Proportion (95% CI) that reported having symptoms in office (same or better at home) all 4 weeks.

Symptom	**Summer**	**Winter**	**All**
Respiratory symptoms	0.467 (0.436, 0.498)	0.495 (0.459, 0.530)	0.480 (0.456, 0.504)
Shortness of breath	0.043 (0.035, 0.054)	0.048 (0.038, 0.060)	0.045 (0.039, 0.054)
Chest tightness	0.037 (0.030, 0.047)	0.043 (0.033, 0.056)	0.040 (0.034, 0.048)
Cough	0.110 (0.095, 0.127)	0.145 (0.124, 0.168)	0.126 (0.113, 0.141)
Sinus congestion	0.294 (0.270, 0.320)	0.345 (0.315, 0.376)	0.319 (0.300, 0.339)
Sneezing	0.219 (0.195, 0.244)	0.231 (0.203, 0.262)	0.224 (0.206, 0.244)
Sore throat	0.127 (0.110, 0.145)	0.131 (0.113, 0.151)	0.129 (0.117, 0.142)
Wheezing	0.045 (0.037, 0.056)	0.041 (0.033, 0.051)	0.043 (0.037, 0.050)
Neurological symptoms	0.589 (0.557, 0.621)	0.585 (0.550, 0.619)	0.587 (0.564, 0.611)
Difficulty concentrating	0.125 (0.107, 0.147)	0.125 (0.109, 0.142)	0.125 (0.112, 0.139)
Dizziness	0.058 (0.048, 0.070)	0.058 (0.047, 0.071)	0.058 (0.050, 0.066)
Fatigue	0.291 (0.263, 0.319)	0.253 (0.224, 0.285)	0.272 (0.252, 0.294)
Headache	0.243 (0.224, 0.276)	0.243 (0.221, 0.267)	0.246 (0.229, 0.264)
Tension/Irritability	0.233 (0.209, 0.259)	0.210 (0.188, 0.235)	0.222 (0.205, 0.240)
Nausea	0.057 (0.046, 0.070)	0.053 (0.042, 0.065)	0.055 (0.047, 0.064)
Tired/strained eyes	0.328 (0.300, 0.356)	0.347 (0.317, 0.379)	0.337 (0.317, 0.358)
Dry eyes	0.327 (0.301, 0.354)	0.316 (0.289, 0.345)	0.322 (0.303, 0.342)
Dry skin	0.162 (0.142, 0.183)	0.273 (0.244, 0.304)	0.216 (0.195, 0.237)

Among respondents, 48.0% overall reported having a respiratory symptom at the office and was the same or better at home (46.7% summer, 49.5% winter). Overall, respiratory symptoms ranged from 4.0% (chest tightness) to 31.9% (sinus congestion). Among respondents, sinus congestion was the most common reported symptom in either summer (29.4%) or winter (34.5%). One in five respondents experienced sneezing in the workplace (22.4% overall, 21.9% summer, 23.1% winter). Chest tightness and wheezing were the least reported symptom overall

(4.0% and 4.3% respectively). Sore throat was reported in 12.9% overall, followed by cough (12.6%) and shortness of breath (4.5%). Other than wheezing, the reported respiratory symptoms were slightly higher in winter than in summer.

One or more neurological symptoms were reported by 58.7% of questionnaire respondents (58.9% summer, 58.5% winter). Overall, tired/strained eyes were the most common of the reported neurological symptoms (33.7%) and most common in both summer (32.8%) and winter (34.7%). Between 20-30% of all respondents reported fatigue, headache, or tension/irritability during both the summer and winter test periods. Dizziness and nausea were the least common of the neurological symptoms (5.8%, 5.5% overall, respectively). Dry eyes and dry skin were reported by 32.2% and 21.6% of respondents overall. Dry skin was notably higher among winter respondents than among those reporting the condition in the summer (27.3% compared to 16.2%).

The proportions and 95% confidence intervals for questionnaire respondents reporting *worse* symptoms in the study office buildings (better at home) are shown in Table 16.

Table 16. Proportion (95% CI) that reported having symptoms in office (better at home) all 4 weeks.

Symptom	**Summer**	**Winter**	**All**
Respiratory symptoms	0.258 (0.233, 0.285)	0.264 (0.229, 0.303)	0.261 (0.239, 0.284)
Shortness of breath	0.018 (0.012, 0.027)	0.020 (0.014, 0.030)	0.019 (0.015, 0.025)
Chest tightness	0.024 (0.018, 0.032)	0.023 (0.015, 0.035)	0.024 (0.018, 0.030)
Cough	0.049 (0.039, 0.062)	0.056 (0.044, 0.071)	0.053 (0.044, 0.062)
Sinus congestion	0.122 (0.106, 0.140)	0.140 (0.115, 0.169)	0.131 (0.115, 0.147)
Sneezing	0.112 (0.093, 0.133)	0.124 (0.101, 0.152)	0.118 (0.102, 0.135)
Sore throat	0.076 (0.062, 0.092)	0.063 (0.052, 0.077)	0.069 (0.060, 0.080)
Wheezing	0.023 (0.017, 0.030)	0.017 (0.012, 0.024)	0.020 (0.016, 0.025)
Neurological symptoms	0.433 (0.405, 0.461)	0.424 (0.392, 0.456)	0.428 (0.407, 0.450)
Difficulty concentrating	0.053 (0.043, 0.066)	0.048 (0.038, 0.061)	0.051 (0.043, 0.059)
Dizziness	0.034 (0.026, 0.044)	0.036 (0.028, 0.046)	0.035 (0.029, 0.042)
Fatigue	0.156 (0.137, 0.176)	0.148 (0.128, 0.171)	0.152 (0.138, 0.167)
Headache	0.155 (0.138, 0.174)	0.157 (0.138, 0.178)	0.156 (0.143, 0.170)
Tension/Irritability	0.168 (0.146, 0.192)	0.157 (0.138, 0.178)	0.162 (0.148, 0.178)

Table 16 continued			
Nausea	0.031 (0.025, 0.039)	0.026 (0.019, 0.036)	0.029 (0.024, 0.035)
Tired/strained eyes	0.226 (0.205, 0.250)	0.236 (0.210, 0.263)	0.231 (0.214, 0.249)
Dry eyes	0.186 (0.165, 0.210)	0.195 (0.171, 0.222)	0.191 (0.174, 0.208)
Dry skin	0.046 (0.036, 0.058)	0.053 (0.041, 0.069)	0.050 (0.041, 0.059)

Overall, 26.1% of the participants reported a respiratory symptom (25.8% summer, 26.4% winter). Sinus congestion (13.1%) and sneezing (11.6%) were the most common reported respiratory symptoms overall, and were slightly more prevalent in winter than in summer. Shortness of breath (1.9%), wheezing (2.0%) and chest tightness (2.4%) were the least common reported symptoms. Sore throat and cough were reported by 6.9% and 5.3%, respectively.

Neurological symptoms were reported by 42.8% of respondents experiencing worse symptoms at work (43.3% summer, 42.4% winter). Tired/strained eyes were the most common symptom (23.1% overall) and was the most common reported in both summer and winter. (22.6%, 23.6% respectively). Nausea was the least reported overall symptom (2.9%). Tension/irritability were reported by 16.2% of respondents overall, followed by headache (15.6%) and fatigue (15.2%). Difficulty concentrating was reported by 5.1% and dizziness was experienced by 3.5%. Among all neurological symptoms, none were notably different between the cohorts reporting during summer or winter. Dry eyes were experienced by 19.1% of respondents overall, and 5.0% reported having dry skin.

Association of Fungal Concentrations and respiratory Symptoms

Association of fungal concentration with worse respiratory symptoms in the office (i.e. better at home) for the duration of the questionnaire period are shown in Table 17. Odds ratios were determined using the logistic regression main effects model. Association between average indoor air and worse respiratory symptoms in the office (OR 0.94, 95% CI (0.82, 1.08), p = 0.378) and maximum indoor and worse respiratory symptoms (OR 0.95, 95% CI (0.84, 1.07), p

= 0.408) did not reach significance, and neither measure displayed interaction effects between season or respiratory condition. Association between outdoor air fungal concentration and worse respiratory symptoms in the office did not reach significance (OR 1.02, 95% CI (0.91, 1.15), p = 0.745). However, interaction effects between outdoor air fungal concentration and winter (p = 0.067) or between outdoor air fungal concentration and respiratory condition (p = 0.080) approached significance.

Table 17. Association of fungal concentration with worse respiratory symptoms in office (better at home) for all 4 weeks.

Variable	Main effects model		With interaction effects					
			Season*variable			Respiratory condition*Variable		
	Odds Ratio* (95% CI)	p-value	Season	Odds Ratio* (95% CI)	p-value	Respir-atory condition	Odds Ratio* (95% CI)	p-value
Fungal air								
Average Indoor	0.94 (0.82, 1.08)	0.378	No significant interaction		0.572	No significant interaction		0.452
Maximum Indoor	0.95 (0.84, 1.07)	0.408	No significant interaction		0.966	No significant interaction		0.562
Outdoor	1.02 (0.91, 1.15)	0.746	Summer	1.1 (0.94, 1.29)	0.273	With	1.08 (0.95, 1.22)	0.256
			Winter	0.86 (0.74, 1.01)	0.067	Without	0.88 (0.77, 1.01)	0.08

*odds ratio is per +1 natural log, computed from logistic regression that included the concentration variable and covariates such as season, respiratory condition, history of tobacco use, and building characteristics (smoking allowed in building, past and current water damage, hours building occupied, ventilation hours, and CO2 output)

Association of Fungal Concentration with Physician Diagnosis of Adverse Respiratory Condition

In the present study, the association of indoor airborne fungi and the reported condition of "have been diagnosed" with respiratory condition (asthma, hay fever, dust allergy, mold allergy, or chemical sensitivity) were determined (Table 18). Odds Ratios were computed from logistic regression using a main effects model which included the concentration variable and covariates such as season, years worked in building, history of tobacco use, and building

characteristics (smoking allowed in building, past and current water damage, hours building occupied, ventilation hours, CO_2 output). Airborne fungal concentrations were categorized separately by average indoor air, maximum indoor air, and outdoor air. There was an association between average indoor airborne fungal concentration and a response of "have been diagnosed" with a respiratory condition (OR 1.13; 95% CI (1.04, 1.23); p= 0.011). In addition, maximum indoor airborne fungal concentration was also associated with the "have been diagnosed" respiratory condition (OR 1.12; 95% CI (1.02, 1.23); p=0.025). Outdoor airborne fungi concentration was not associated with the "have been diagnosed" condition (OR 1.06; 95% CI (0.99, 1.14); p=0.127). Interestingly, the study data suggests occupational indoor airborne fungal exposure in the workplace may be a greater contributor to respiratory conditions than outdoor exposure.

In some instances, one explanatory variable will have an effect on an outcome response depending on a second explanatory variable. In the present study, interaction effects for season (winter, summer) and Years Worked in Building (<5 years, ≥5 years) were added to the main effects model and subsequently analyzed. Two variables (Winter and ≥5 years) each influenced the positive association between airborne fungal concentration and a reported respiratory health effect (i.e., reported as "have been diagnosed") *as well as* outdoor airborne fungal concentration and respiratory condition. The variables Summer and <5 years did not appear to significantly influence the association between indoor or outdoor airborne fungal concentration and a diagnosed respiratory condition.

Table 18. Association of fungal concentration with "have been diagnosed" with a respiratory condition (asthma, hayfever, dust allergy, mold allergy, or chemical sensitivity).

Variable	Main effects model		With interaction effects					
			Season*variable			(Years Worked in Building)*Variable		
	Odds Ratio* (95% CI)	p-value	Season	Odds Ratio* (95% CI)	p-value	Years in Building	Odds Ratio* (95% CI)	p-value
Fungal air								
Average Indoor	1.13 (1.04, 1.23)	0.011	Summer	1.14 (0.96, 1.35)	0.137	<5 years	1.07 (0.96, 1.19)	0.223
			Winter	1.14 (1.05, 1.23)	0.018	≥5 years	1.21 (1.07, 1.37)	0.004
Maximum Indoor	1.12 (1.02, 1.23)	0.025	Summer	1.1 (0.91, 1.31)	0.326	<5 years	1.06 (0.95, 1.19)	0.286
			Winter	1.15 (1.06, 1.25)	0.012	≥5 years	1.19 (1.05, 1.35)	0.011
Outdoor	1.06 (0.99, 1.14)	0.127	Summer	0.98 (0.87, 1.11)	0.781	<5 years	1.01 (0.93, 1.10)	0.836
			Winter	1.12 (1.03, 1.22)	0.025	≥5 years	1.09 (1.00, 1.18)	0.056

*odds ratio is per +1 natural log, computed from logistic regression that included the concentration variable and covariates such as season, years worked in building, history of tobacco use, and building characteristics (smoking allowed in building, past and current water damage, hours building occupied, ventilation hours, and CO2 output)

Association of Fungal Concentration and reported Neurological Symptoms

The association of fungal concentration and reported *worse* neurological symptoms in the office (i.e., better at home) was assessed for the four-week period prescribed in the BASE questionnaire (Table 19). Odds ratios were calculated from the logistic regression main effects model which included the concentration variable and covariates (season, respiratory condition, history of tobacco use, and building characteristics (smoking allowed, past and current water damage, occupied hours, ventilation hours, CO_2 output). Airborne fungal concentrations were further categorized by average indoor, maximum indoor, and outdoor measures. There were no

detected associations between average indoor and worse neurological symptoms experienced in the study buildings ((OR 1.03, 95% CI (0.94, 1.13), p = 0.532). Further, no interaction effects for season or respiratory condition variables were found (p = 0.452, p = 0.310 respectively).

Table 19. Association of fungal concentration with worse neurological symptoms in office (i.e., better at home) for all 4 weeks.

Variable	**Main effects model**			**With interaction effects**					
				Season*variable			**Respiratory condition*Variable**		
	Odds Ratio*		**p-value**		**Odds Ratio***	**p-value**		**Odds Ratio***	**p-value**
	(95% CI)			**Season**	**(95% CI)**		**Respiratory condition**	**(95% CI)**	
Fungal air									
Average Indoor	1.03	(0.94, 1.13)	0.532	No significant interaction		0.452	No significant interaction		0.31
Maximum Indoor	1.02	(0.93, 1.13)	0.623	No significant interaction		0.164	No significant interaction		0.215
Outdoor	1.03	(0.95, 1.12)	0.48	No significant interaction		0.632	No significant interaction		0.71

*odds ratio is per +1 natural log, computed from logistic regression that included the concentration variable and covariates such as season, respiratory condition, history of tobacco use, and building characteristics (smoking allowed in building, past and current water damage, hours building occupied, ventilation hours, and CO2 output)

Association between maximum indoor fungal concentration and worse neurological symptoms were not significant (OR 1.02, 95% CI (0.93, 1.13), p = 0.623), and no interaction effects were detected between outdoor concentration and worse neurological symptom in the office (OR 1.03, 95% CI (0.95, 1.12), p = 0.480. No interactions for season or respiratory condition variables were indicated.

Respiratory Symptoms and Season

Scatter plots were constructed comparing the proportion of questionairre respondents (y axis) in each of the BASE Study buildings (summer, n = 52; winter, n = 48) versus natural log

mean, maximum, and outdoor concentrations of airborne fungi (x axis) for each respective building in order to visualize potential trends (Figures 8-13). The cross-sectional design limited establishment of causal relationships, but was useful in examining differences between self-reported respiratory conditions and seasonal effects, comparisons between mean and maximum airborne fungal concentrations, and differences between indoor and outdoor measures. Greater variability was observed in the relationships between reported respiratory conditions and fungal concentrations (both indoor and outdoor) in winter months than in summer months.

Figure 8. Scatter plot showing the relationship of questionnaire respondents reporting a respiratory condition versus the mean concentration of airborne fungi (summer, n = 52 buildings).

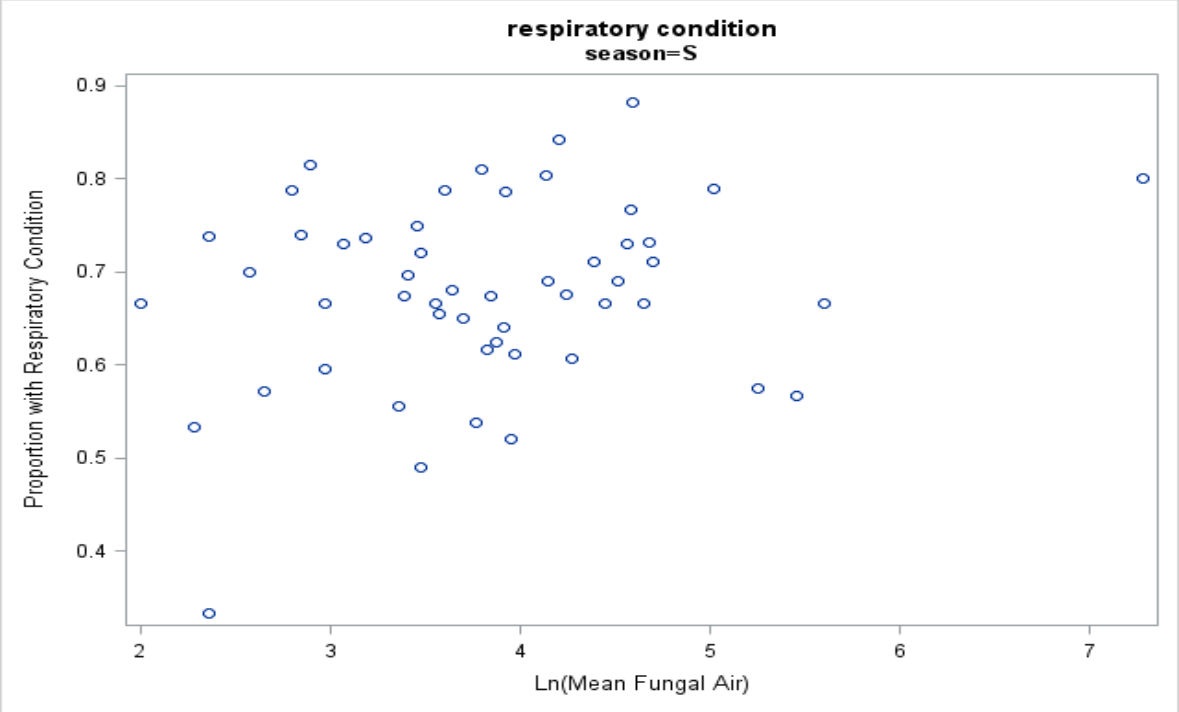


Figure 9. Scatter plot showing the relationship of questionnaire respondents reporting a respiratory condition versus the mean concentration of airborne fungi (Winter, n = 48 buildings).

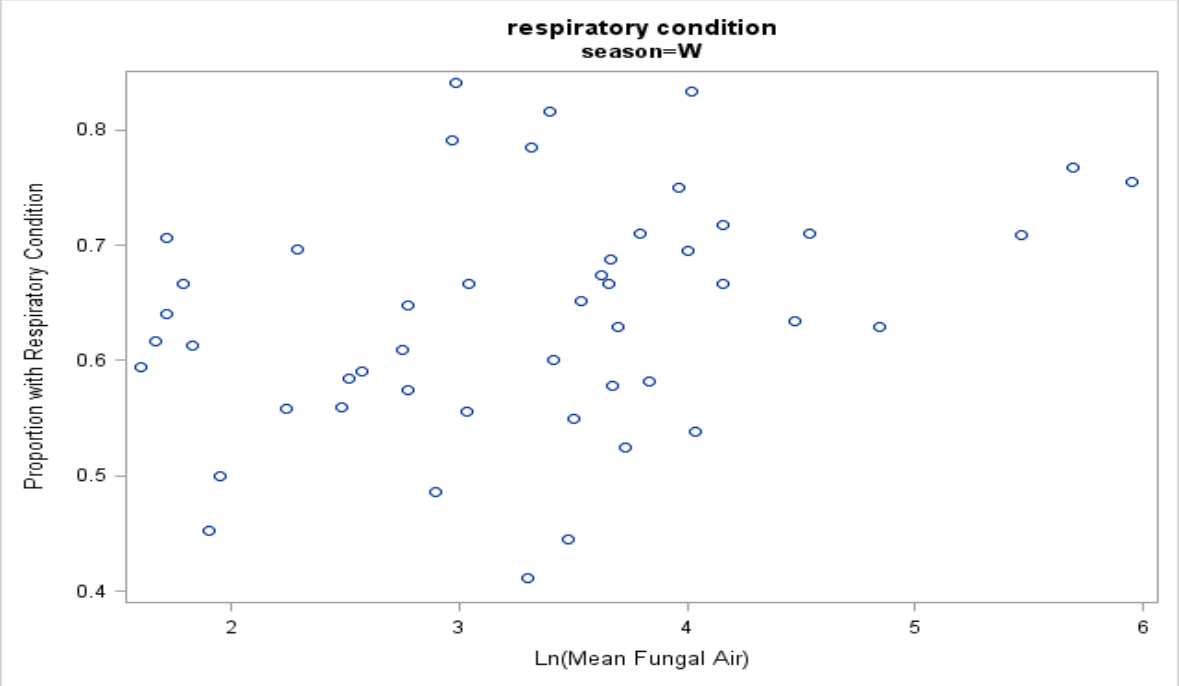


Figure 10. Scatter plot showing the relationship of questionnaire respondents reporting a respiratory condition versus the maximum concentration of airborne fungi (Summer, n = 52 buildings).

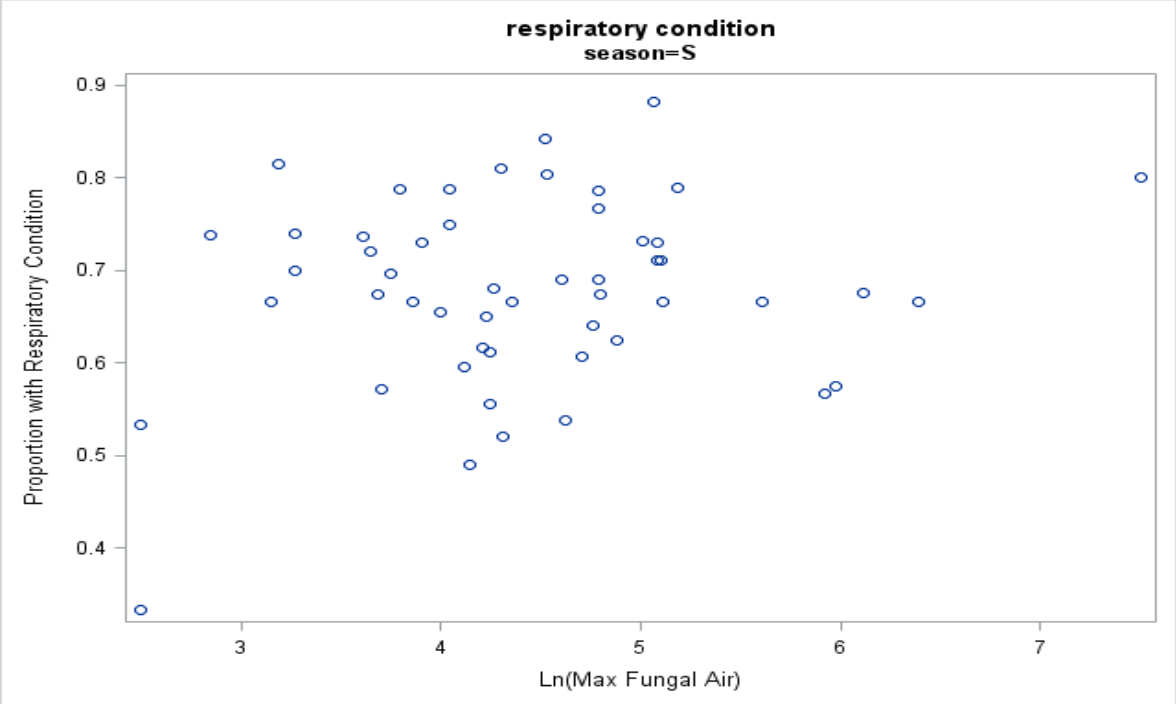


Figure 11. Scatter plot showing the relationship of questionnaire respondents reporting a respiratory condition versus the maximum concentration of airborne fungi (Winter, n = 48 buildings).

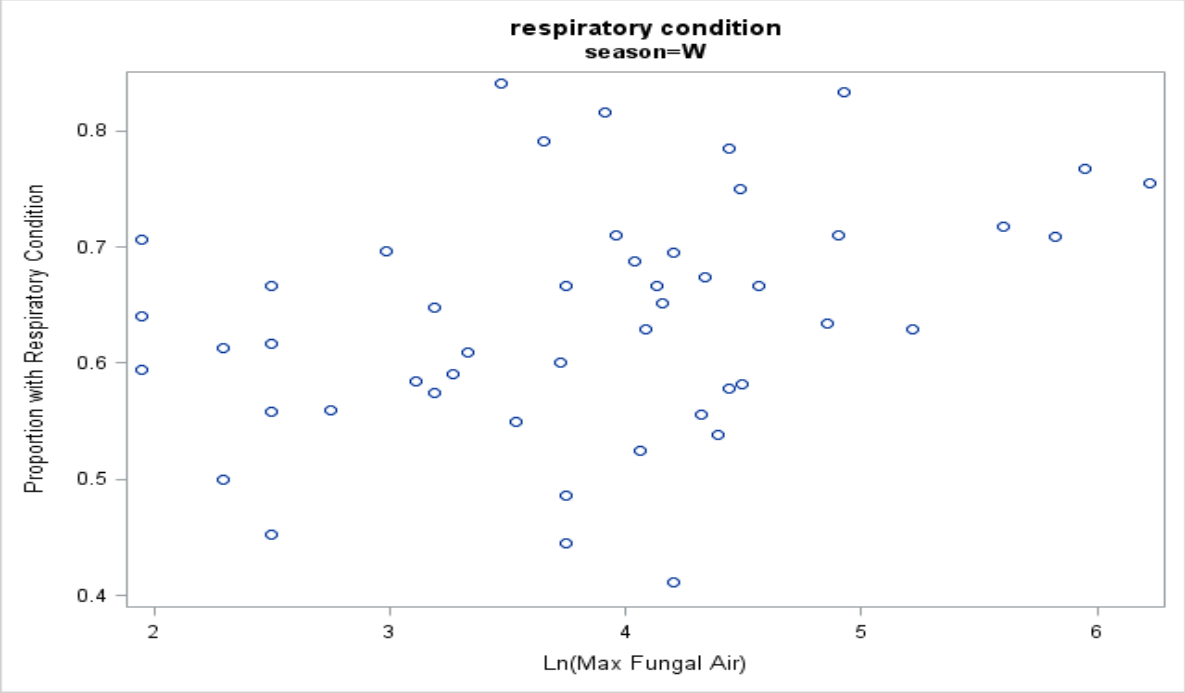


Figure 12. Scatter plot showing the relationship of questionnaire respondents reporting a respiratory condition versus the outdoor concentration of airborne fungi (Summer, n = 52 buildings).

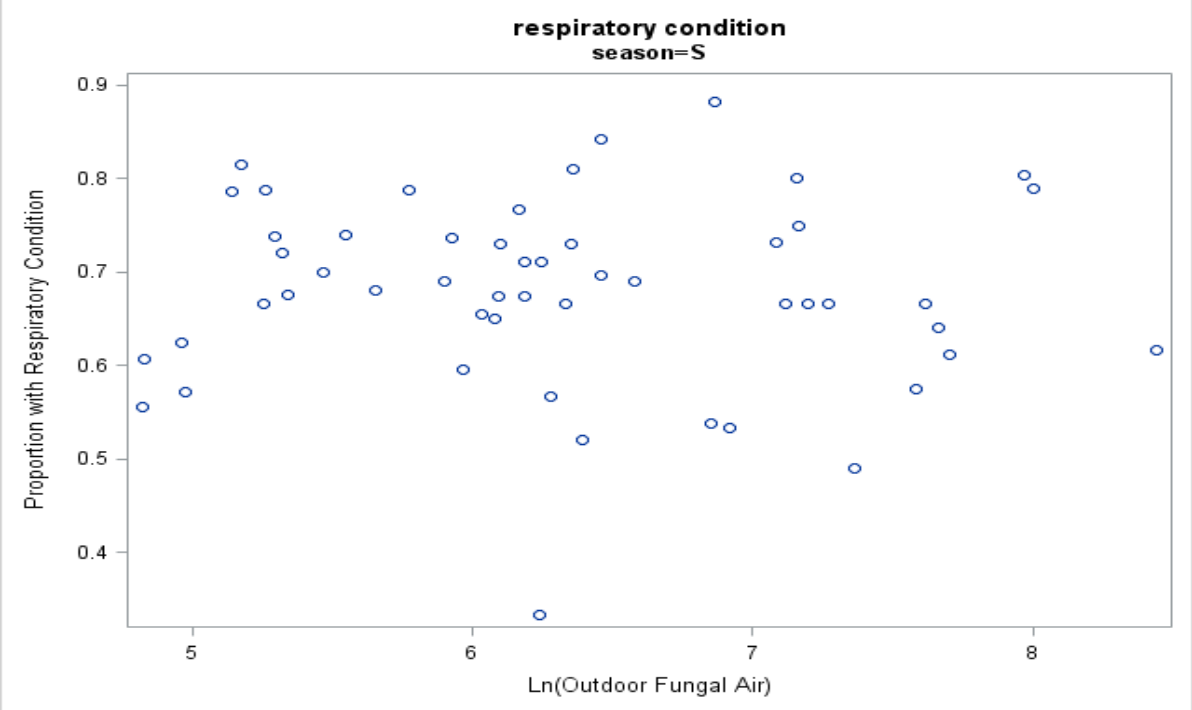
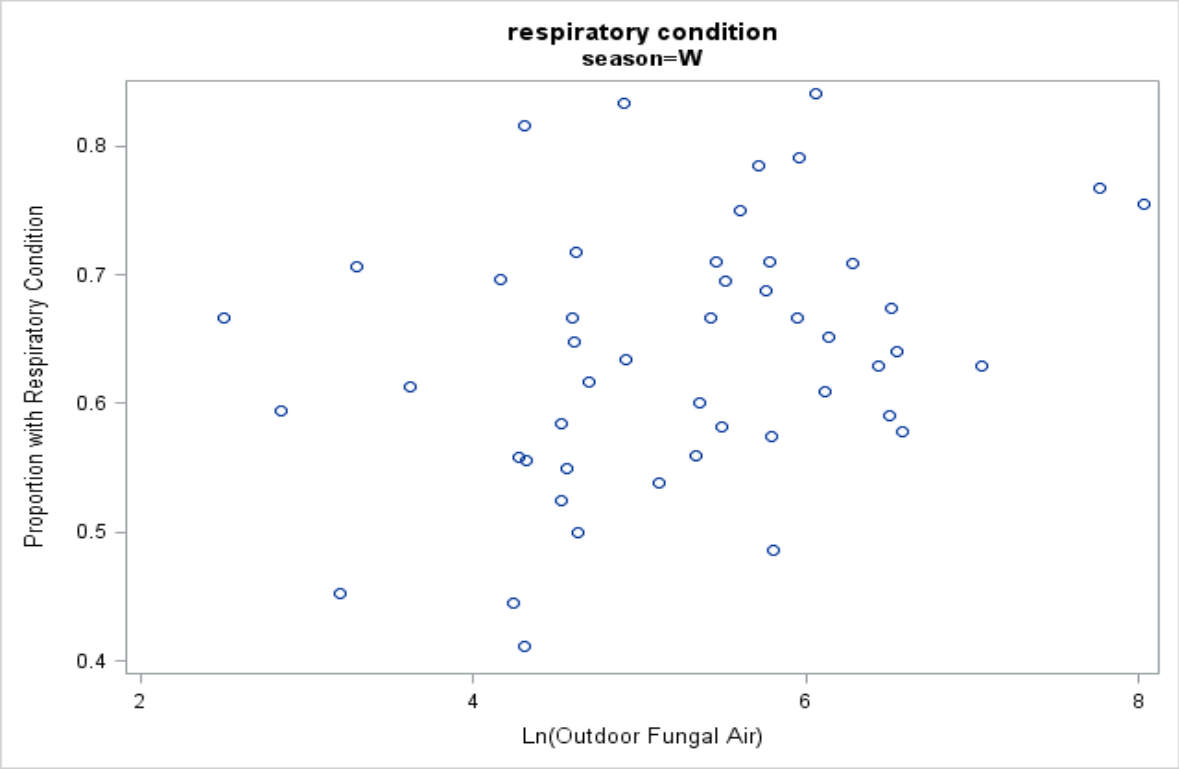


Figure 13. Scatter plot showing the relationship of questionnaire respondents reporting a respiratory condition versus the outdoor concentration of airborne fungi (Winter, n = 48 buildings).



CONCLUSION

The BASE Study provides normative demographic and health data from occupants working in observed spaces in 100 study buildings located in ten climatic zones encompassing the continental United States. The comprehensive nature of this large cross-sectional study makes it a valuable source of reference data in the study of culturable airborne fungi as a measure of IAQ and health perceptions of occupants.

Although comprehensive, the BASE Study had potential limitations, including differential distribution of included buildings across the climate zones. The randomized selection process of buildings was intended to select representative examples in U.S. building stock. However, recognition of potential selection bias should be noted. Buildings publicized as

"problem" or "sick" were excluded, and all buildings that were eventually selected for inclusion required owner/manager approval beforehand. Further, work spaces with occupancies of at least fifty people limited study to larger office buildings and necessitated taking clustering effects of occupants' health responses into account.

The present study examined descriptive comparisons of questionnaire responses and culturable airborne fungi, but causal relationships were not definitive in the cross-sectional design. However the large number of participants (n = 4326), comprehensive data collection, large number of buildings (n = 100), and continental scope are strengths of the BASE Study in comparison to smaller longitudinal studies with respect to generalizability.

In the present study, there were observed associations between a questionnaire response of "have been diagnosed" with a respiratory condition and both indoor and maximum indoor airborne fungal concentrations. Outdoor airborne fungi was not associated with a response of "have been diagnosed." The variables "Winter" and "≥ 5 years worked in building" each influenced the positive association between reported respiratory symptom and both indoor and outdoor airborne fungal concentrations.

There were no detected associations between mean or maximum indoor airborne fungal concentrations and worse neurological symptoms, and no observed interaction effects for season, respiratory condition, or outdoor concentration. The data suggest respiratory conditions are more heavily influenced by presence of airborne fungi, and that long-term exposure may increase sensitivity to exposures. Longitudinal studies will be useful in establishing causal relationships.

CHAPTER 5: FUTURE RESEARCH NEEDS

The potential avenues for future studies are numerous. Within selected climatic regions, longitudinal monitoring (fewer buildings but concentrated focus) should be performed in order to detect seasonal changes in the microbiome within a common space. Coupled with current molecular methods, the identification of of fungi detected in buildings can be defined to the species level, providing a more precise understanding of exposure among building occupants. In addition, present-day climatic zones (if different) should be compared to BASE climatic zones to determine if heating or air conditioning requirements are changing over time as climate changes. These should be coupled with identification of species among the buildings in those regions to determine if microbiomes are being affected.

In a recent study, Vesper and colleagues (Vesper et al., 2018) evaluated the Environmental Relative Moldiness Index (EMRI) for possible application in multi-level office building settings. The EMRI has historically been used to quantify mold contamination and water damage problems in private homes for studies of asthma (Vesper and Wymer, 2016). It was developed by the EPA in conjunction with the U.S. Department of Housing and Urban Development to analyze indoor presence of 36 indicator molds either commonly associated with water damage or which are found indoors irrespective of water damage (Vesper et al., 2007). The application of this index to office buildings presents potential opportunities for future studies, particularly with respect to the investigation of effects on occupant health when quantified presence of specific mold species is determined. The potential incorporation of EMRI in future studies will be considered.

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