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Cle Elum–Roslyn High School
Cle Elum, Washington

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PREFACE

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Health Hazard Evaluation Report 97–0045–2676
Cle Elum–Roslyn High School
Cle Elum, Washington
March 1998

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SUMMARY

On May 8, 1997, the National Institute for Occupational Safety and Health (NIOSH) received a request from the Kittitas County Health Department for technical assistance in the evaluation of potential microbial contamination in the Cle Elum–Rosyln High School, Cle Elum, Washington. A similar request had been previously received by NIOSH from employees of the school. On May 28–29,1997, NIOSH investigators conducted an initial visit to the school. Bulk samples of building materials were collected to assess potential areas of microbial contamination and measurements were made of general indoor air quality comfort parameters. On September 15–16, 1997, NIOSH investigators conducted a follow–up evaluation during which air samples were collected for culturable fungi and spores, and measurements were repeated for general indoor air quality parameters.

Fungal concentrations from the bulk material samples ranged from none detected (ND) to $6.4x10^6$ colony forming units per gram of material (CFU/gm). The predominant fungi identified included *Aspergillus versicolor*, *Chaetomium*, *Penicillium*, *Paecilomyces*, *Stachybotrys chartarum* (a.k.a., *S. atra*), *Rhodotorula*, *Sporobolomyces*, and unidentified yeasts. Most of the bulk material samples revealed low concentrations of fungi that are not consistent with the conclusion that an active microbial reservoir exists. However, seven of the twenty bulk material samples showed high fungal concentrations and/or were identified with significant genera. Bacterial concentrations from the bulk material samples ranged from ND to $1.6x10^7$ CFU/gm (the highest concentrations were found in duct insulation and ceiling tiles). Gram negative bacteria were the major species detected and are normally found in association with large amounts of moisture. Microbiologic analysis of nine debris and dirt samples indicated fungal levels ranging from 344 to $2.6x10^6$ CFU/gm and bacterial concentrations ranging from $1.1x10^4$ to $>5.4x10^7$ CFU/gm (the highest concentrations were observed in the crawlspace dirt). The predominant fungal genera identified include *Acremonium*, *Penicillium*, *Cladosporium*, and unidentified yeasts; the predominant bacterial type were Gram negative species. Although in low concentrations, *Aspergillus versicolor* and *Stachybotrys chartarum* (mycotoxin producers) were identified in the crawlspace dirt.

The geometric mean of airborne fungal concentration at various locations inside the building ranged from 99 colony forming units per cubic meter of air ([CFU/m^3] (geometric standard deviation of 1.2) in the faculty lounge to >1492 CFU/m^3 (geometric standard deviation of 1.1) in the crawlspace. Acceptable levels of airborne microorganisms have not been established, primarily due to the varying immunogenic susceptibilities of individuals. Airborne dissemination [characterized by elevated levels in the complaint area, compared to outdoor and non–complaint areas, and an anomalous ranking among the microbial species] correlated to occupant symptomatology may suggest that the contaminant may be responsible for the health effects. Outside of the building, the geometric mean fungal concentration was 365 CFU/m^3 (geometric standard deviation of 2.2). All of

the indoor fungal concentrations were below the outdoor geometric mean level. The taxonomic ranking of many of the indoor locations was dissimilar to the ranking observed outdoors. Specifically, a higher percentage of *Penicillium* sp. were identified in some locations in the building. Greater relative numbers of spores were observed for the samples collected outdoors compared with indoor sites. The most significant event observed from the spore samples was the identification of *Stachybotrys* spores from the crawlspace sample. Although the relative concentration is low, the finding is consistent with the bulk sample results which showed the presence of culturable *Stachybotrys* in samples collected from the exterior duct lining of an air handling unit and in the soil within the crawlspace.

Carbon dioxide concentrations, temperatures, and relative humidity readings were generally found to be within those recommended by the American Society of Heating Refrigeration and Air-Conditioning Engineers (ASHRAE).

Based on the information and data obtained during this Health Hazard Evaluation, NIOSH investigators conclude that substantial microbial contamination existed in the crawlspace which would not make it a suitable location for placement of air handling units for the building. Recommendations related to the general ventilation systems, building cleaning, and dealing with water incursion incidents are included in the report.

Keywords: SIC 8211 (Elementary and Secondary Schools), moisture incursion, microbial contamination, fungi, spores, bacteria, *Stachybotrys*, *Aspergillus*, *Penicillium*, indoor air quality, ventilation

TABLE OF CONTENTS

INTRODUCTION

On May 8, 1997, the National Institute for Occupational Safety and Health (NIOSH) received a letter from the Kittitas County Health Department requesting technical assistance in evaluation of potential microbial contamination in the Cle Elum–Rosyln High School, Cle Elum, Washington. A similar request had been previously received by NIOSH from employees of the school.

On May 28–29,1997, NIOSH investigators (composed of an Industrial Hygienist and an Industrial Hygiene Engineer) conducted an initial visit to the Cle Elum–Rosyln High School. An opening conference was held with representatives of the school district, state and local health department, state labor agency, teachers, and other involved groups. Information was obtained related to the building and the history of the concerns with microbiological contamination. A walk–through inspection was made of the building exterior and interior. Critical attention was focused on locations identified as water incursion points and those heating, ventilating, and air–conditioning (HVAC) units located in occupant–reported problem areas and in the building crawlspace. Bulk samples from various building materials and crawlspace soil were collected to assess potential areas of microbial contamination and measurements were made of general indoor air quality comfort parameters. A closing conference was then held with the aforementioned representatives during which preliminary findings were discussed. A letter report containing the sample results and recommendations was provided to relevant personnel on August 8, 1997.

On September 15–16, 1997, NIOSH investigators conducted a follow–up environmental evaluation at the school. During this visit, air samples were collected for culturable fungi and spores, and measurements were repeated for general indoor air quality parameters. A closing conference was held to discuss the types of samples collected.

BACKGROUND

The high school building was constructed in the 1970's and currently houses approximately 300 students, 19 teachers, and 11 other staff. Employee reports of health complaints began in 1994. The employees reported that their health complaints might be related to microbial contamination, possibly associated with water incursion into the building. There were reportedly two major episodes of water incursion, the first in 1994 during a large–scale renovation of the building. Temporary roof coverings, which were being used during roof reconstruction, reportedly became displaced allowing rain to enter portions of the building. This resulted in water damage to ceiling tiles and walls. Reports from teachers indicated that there were areas of visible mold growth on building surfaces following the leakage. The second major instance of moisture incursion was during the winter of 1996 – 1997 when an unusually heavy snowfall led to the formation of an “ice dam” around the building. When the snow melted, water drained toward the school building, entering the crawlspace below the building. At that time, water was flowing in and along the outside air ducts for two air handling units located in the crawlspace below the D wing, due to the below grade location of the outside air intakes. Prior to the NIOSH visits, these two units were shut down and remained inoperable during the period of the NIOSH evaluation.

The building ventilation system consists of combinations of 14 central package air handling units (AHU), 9 unit ventilators, and 6 fin/tube (radiant heat) systems. The fin/tube units are primarily located in the central hallways. The air in the A and B wing classrooms is conditioned with unit ventilator systems with the exception of Room 201, which is served by AHU 3. The D wing classrooms are all served by AHUs with the exception of the Art Room which has a unit ventilator. All of the systems are connected to a central computer control system which regulates the temperature and the amount of introduced outdoor air. All of the package AHUs are located within the

building envelope except for AHU 12 and AHU 13 which are located in the crawlspace beneath the D wing (the units which had been shutdown). The package AHUs use ducted returns with thermal insulation located on the exterior of the duct surface. Filtration for the package AHUs consists of medium efficiency pleated filters. The unit ventilators used low efficiency (less than 20%) fiber media.

METHODS

Initial Survey

Visual Inspection

A visual inspection was made of the building exterior and interior. Critical attention was focused on specific locations identified as water incursion points and those HVAC units located in occupant reported problem areas and in the building crawlspace. Visual inspection of interior return air duct surfaces was facilitated with a rigid boroscope (Instrument Technologies, Inc., Westfield, Massachusetts).

Bulk Samples

Twenty–nine bulk material or debris samples were collected in those locations that were suspected of microbial contamination (based on visible observation) or those locations that provided an environment conducive to the growth of microorganisms. Collection of 20 bulk samples from interior duct insulation and floor carpet was facilitated by cutting an approximate one square inch section from the material. Nine samples of debris from supply air diffusers, and dirt from the D wing crawlspace were also collected and placed into glass vials. Representative portions of each sample were weighed and vortexed in a recorded volume of 0.2% Tween 20. Serial dilutions of the prepared samples were then plated to the appropriate nutrient media. The nutrient media used for fungi included malt extract agar (MEA) and cornmeal agar (CMA); the nutrient media used for bacterial cultures was tryptic soy agar (TSA). MEA and TSA are general nutrient media used for the enumeration of fungi and bacteria, respectively. CMA is a selective media to promote the growth of *Stachybotrys* species. Additionally, a single "sticky" tape sample was collected of a suspect fungal colony by using the adhesive side of the tape to pull spore structures and hyphae from the growth surface. The tape sample was mounted (in the field) to a glass slide and subsequently microscopically analyzed.

Comfort Indices

In addition to collecting the bulk samples for microbial contamination, indicators of occupant comfort were measured in each room and outdoors. These indicators were carbon dioxide (CO_2), temperature, and relative humidity (RH). Real–time CO_2 concentrations were measured using a Gastech Model RI–411A, portable CO_2 indicator. This portable, battery–operated instrument uses a non–dispersive infrared absorption detector to measure CO_2 in the range of 0–4975 parts per million (ppm), with a sensitivity of ±25 ppm. Real–time temperature and humidity measurements were made using a TSI Incorporated VelociCalc Plus, Model 8360, battery–operated air velocity meter. This meter is capable of providing direct readings for dry–bulb temperature and RH, ranging from 14 to 140°F +/– 0.5°F and 20 to 95% +/– 4%, respectively.

Follow–up Survey

Air Samples for Fungi (culturable and spores)

To determine the concentrations of culturable airborne fungi, the Anderson single–stage viable cascade impactor was used at a calibrated flow rate of 28.3 liters per minute (lpm). All culturable samples were collected over a sample time of 10 minutes (with the exception of the crawlspace samples, which were collected at 5 to 7 minute intervals to compensate for anticipated heavier fungal loads). Malt extract agar (MEA) and dichloran glycerol agar (DG18) were used for the

enumeration of fungi (DG18 is used as the nutrient agar to select for xerophilic species). All sample plates were incubated at room temperature (approximately 25˚C). The taxa and rank of the collected microorganisms were determined by morphological characteristics.

Air samples for culturable fungi were collected at eight interior building locations and one outdoor location. Sampling locations included the Computer Lab, Room 100, Spanish (Room 102), Business (Room 300), Home and Family (Room 302), Computer Aided Design (CAD, Room 303), the faculty lounge, and the crawlspace under the D wing. At each sample location, four replicate samples of each nutrient media were collected for culturable fungi (with the exception of the outdoor and crawlspace locations where seven and five replicate samples were collected, respectively). Samples were collected over a two day period.

To measure the airborne concentrations of total spores, 13 area air samples (3 of which are duplicate locations) were collected at locations throughout the building, as well as an outdoor sample location (replicated on the second day). The sample locations included CAD, Computer Lab, Business, Room 100, Home and Family, Spanish, the faculty lounge, and the crawlspace (duplicate locations included the Computer Lab, Business, and Home and Family). Spores were collected with 37 millimeter (mm) mixed cellulose ester filters. The filters were placed on cellulose support pads and sealed in plastic filter cassettes. The filter holders were connected via Tygon™ tubing to Gillian Hi Flow Sampler™ battery–operated personal sampling pumps operating at a flow rate of 2 liters per minute (lpm) over an 8–hour period. Calibration of the flow rates was performed immediately prior to, and after, sampling. For subsequent calculation of sample volumes, the mean of the pre– and post– sampling flow rates was used. Calibration of the pumps on–site was accomplished with a rotometer, which in turn was calibrated with a primary standard (bubble flowmeter) prior to the evaluation. Samples were analyzed for fungal spore counts by optical microscopy. Filters were cleared with acetone vapor, mounted in cotton blue/lactic acid, and scanned at 400x magnification with bright field or phase contrast illumination. Two hundred fields were counted for each sample. Only particles greater than 2 μm in diameter were considered to be possible fungal spores.

Comfort Indices

In addition to collecting air samples, indicators of occupant comfort were measured in each of the rooms of the building and outdoors. These indicators were CO_2 concentration, temperature, and RH, and were collected in accordance with the methods used during the initial survey.

EVALUATION CRITERIA

NIOSH investigators have completed over 1,200 investigations of the occupational indoor environment in a wide variety of non–industrial settings. Almost all of these investigations have been conducted since 1979.

The symptoms and health complaints reported to NIOSH by building occupants have been diverse and usually not suggestive of any particular medical diagnosis or readily associated with a causative agent. A typical spectrum of symptoms has included headaches, unusual fatigue, varying degrees of itching or burning eyes, irritations of the skin, nasal congestion, dry or irritated throats, and other respiratory irritations. Typically, the workplace environment has been implicated because workers report that their symptoms lessen or resolve when they leave the building.

A number of published studies have reported a high prevalence of symptoms among occupants of office buildings.[1,2,3,4,5] Scientists investigating indoor environmental problems believe that there are multiple factors contributing to building–related occupant complaints.[6,7] Among these factors are imprecisely–defined characteristics of HVAC systems, cumulative effects of exposure to low concentrations of multiple chemical pollutants,

odors, elevated concentrations of particulate matter, microbiological contamination, and physical factors such as thermal comfort, lighting, and noise.[8,9,10,11,12,13] Indoor environmental pollutants can arise from either outdoor sources or indoor sources.

There are also reports describing results which show that occupant perceptions of the indoor environment are more closely related to the occurrence of symptoms than any measured indoor contaminant or condition.[14,15,16] Some studies have shown relationships between psychological, social, and organizational factors in the workplace and the occurrence of symptoms and comfort complaints.[16,17,18,19]

Less often, an illness may be found to be specifically related to something in the building environment. Some examples of potentially building–related illnesses are allergic rhinitis, allergic asthma, hypersensitivity pneumonitis, Legionnaires' disease, Pontiac fever, carbon monoxide poisoning, and reaction to boiler corrosion inhibitors. The first three conditions can be caused by various microorganisms or other organic material. Legionnaires' disease and Pontiac fever are caused by Legionella bacteria. Sources of carbon monoxide include vehicle exhaust and inadequately–ventilated kerosene heaters or other fuel–burning appliances. Exposure to boiler additives can occur if boiler steam is used for humidification or is released by accident.

Problems that NIOSH investigators have found in the non–industrial indoor environment have included the following: poor air quality due to ventilation system deficiencies, overcrowding, volatile organic chemicals from furnishings, emissions from office machines, structural components of the building and contents, tobacco smoke, microbiological contamination, and outside air pollutants; comfort problems due to improper temperature and RH conditions, poor lighting, and unacceptable noise levels; adverse ergonomic conditions; and job–related psychosocial stressors. In most cases, however, these problems could not be directly linked to the reported health effects.

Standards specific for the non–industrial indoor environment do not exist. NIOSH, the Occupational Safety and Health Administration (OSHA), and the American Conference of Governmental Industrial Hygienists (ACGIH®) have published regulatory standards or recommended limits for occupational exposures.[20,21,22] With few exceptions, pollutant concentrations observed in non–industrial indoor environments fall well below these published occupational standards or recommended exposure limits. American Society of Heating Refrigeration and Air–Conditioning Engineers (ASHRAE) has published recommended building ventilation design criteria and thermal comfort guidelines.[23,24] The ACGIH has also developed a manual of guidelines for approaching investigations of building–related complaints that might be caused by airborne living organisms or their effluents.[25]

Measurement of indoor environmental contaminants has rarely proved to be helpful in determining the cause of symptoms and complaints except where there are strong or unusual sources, or a proven relationship between contaminants and specific building–related illnesses. The low–level concentrations of particles and variable mixtures of organic materials usually found are difficult to interpret and usually impossible to causally link to observed and reported health symptoms. However, measuring ventilation and comfort indicators such as CO_2, temperature, and RH, has proven useful in the early stages of an investigation in providing information relative to the proper functioning and control of HVAC systems.

NIOSH and the Environmental Protection Agency (EPA) jointly published a manual on building air quality, written to help prevent environmental problems in buildings and solve problems when they occur.[26] This manual suggests that indoor environmental quality (IEQ) is a constantly changing interaction of a complex set of factors. Four of the most important elements involved in the development of IEQ problems are: (1) a source of odors or contaminants; (2) a problem with the design or operation of the HVAC system; (3) a pathway between the contaminant source and the location of

the complaint; and (4) the building occupants. A basic understanding of these factors is critical to preventing, investigating, and resolving IEQ problems.

The basis for measurements made during this evaluation are listed below.

Microorganisms

Microorganisms (including fungi and bacteria) are normal inhabitants of the environment. The saprophytic varieties (those utilizing non–living organic matter as a food source) inhabit soil, vegetation, water, or any reservoir that can provide an adequate supply of a nutrient substrate. Under the appropriate conditions (optimum temperature, pH, and with sufficient moisture and available nutrients) saprophytic microorganism populations can be amplified. Through various mechanisms, these organisms can then be disseminated as individual cells or in association with soil or dust particles or water droplets. In the outdoor environment, the levels of microbial aerosols will vary according to the geographic location, climatic conditions, and surrounding activity. In a "normal" indoor environment, where there is no unusual source of microorganisms, the level of microorganisms may vary somewhat as a function of the cleanliness of the HVAC system and the numbers and activity level of the occupants. Generally, the indoor levels are expected to be below the outdoor levels (depending on HVAC system filter efficiency) with consistently similar ranking among the microbial species.[27,28]

Some individuals manifest increased immunologic responses to antigenic agents encountered in the environment. These responses and the subsequent expression of allergic disease is based, partly, on a genetic predisposition.[29] Allergic diseases which have been reported to be associated with exposures in indoor environments include allergic rhinitis (nasal allergy), allergic asthma, allergic bronchopulmonary aspergillosis (ABPA), and extrinsic allergic alveolitis (hypersensitivity pneumonitis).[27] Allergic respiratory diseases resulting from exposures to microbial agents have been documented in agricultural, biotechnology, office, and home environments.[30,31,32,33,34,35,36,37]

Symptoms vary with the type of allergic disease: (1) allergic rhinitis is characterized by episodes of sneezing, itching of the nose, eyes, palate, or pharynx, nasal stuffiness with partial or total airflow obstruction, and rhinorrhea with postnasal drainage; (2) allergic asthma is characterized by episodic or prolonged wheezing and shortness of breath due to bronchial narrowing; (3) ABPA is characterized by the production of IgE and IgG antibodies with symptoms of cough (which is sometimes productive of mucous), fatigue, low grade fever, and wheezing.[27,38] Heavy exposures to airborne microorganisms can result in an acute form of extrinsic allergic alveolitis which is characterized by chills, fever, malaise, cough, and dyspnea (shortness of breath) appearing 4 to 8 hours after exposure. Onset of the chronic form of extrinsic allergic alveolitis is thought to be induced by a continuous low–level exposure, and onset occurs without chills, fever, or malaise, but is characterized by progressive shortness of breath with weight loss.[39] However, despite these relatively well–defined diseases which have been reported to occur in office environments, as described previously, symptoms most commonly encountered by office workers are generally not associated with any particular medical diagnosis or etiologic agent.

Acceptable levels of airborne microorganisms have not been established, primarily due to the varying immunogenic susceptibilities of individuals. Relationships between health effects and environmental microorganisms must be determined through the combined contributions of medical, epidemiologic, and environmental evaluation.[25] The current strategy for on–site evaluation involves a comprehensive inspection of problem areas to identify sources of microbial contamination and routes of dissemination. In those locations where contamination is visibly evident or suspected, bulk samples may be collected to identify the predominant species (fungi, bacteria, and thermoactinomycetes). In limited situations, air samples for microorganisms may be collected to document the airborne presence

of a suspected microbial contaminant. Airborne dissemination (characterized by elevated levels in the complaint area, compared to outdoor and non–complaint areas, and an anomalous ranking among the microbial species) correlated to occupant symptomatology may suggest that the contaminant may be responsible for the health effects.

Carbon Dioxide

Carbon dioxide is a normal constituent of exhaled breath and, if monitored, can be used as a screening technique to evaluate whether adequate quantities of outside air are being introduced into an occupied space. ASHRAE's most recently published ventilation standard, ASHRAE 62–1989, Ventilation for Acceptable Indoor Air Quality, recommends outdoor air supply rates of 20 cubic feet per minute per person (cfm/person) for office spaces, and 15 cfm/person for reception areas, classrooms, libraries, auditoriums, and corridors. Maintaining the recommended ASHRAE outdoor air supply rates when the outdoor air is of good quality, and there are no significant indoor emission sources, should provide for acceptable indoor air quality.

Indoor CO_2 concentrations are normally higher than the generally constant ambient CO_2 concentration (range 300–350 ppm). Carbon dioxide concentration is used as an indicator of the adequacy of outside air supplied to occupied areas. ASHRAE Standard 62–1989 recommends 1000 ppm as the upper limit for comfort (odor) reasons.[23] When indoor CO_2 concentrations exceed 800 ppm in areas where the only known source is exhaled breath, inadequate ventilation is suspected.[40] Elevated CO_2 concentrations suggest that other indoor contaminants may also be increased. It is important to note that CO_2 is not an effective indicator of ventilation adequacy if the ventilated area is not occupied at its usual level.

Temperature and Relative Humidity

Temperature and RH measurements are often collected as part of an indoor environmental quality investigation because these parameters affect the perception of comfort in an indoor environment. The perception of thermal comfort is related to one's metabolic heat production, the transfer of heat to the environment, physiological adjustments, and body temperatures.[41] Heat transfer from the body to the environment is influenced by factors such as temperature, humidity, air movement, personal activities, and clothing. The ASHRAE Standard 55–1992, specifies conditions in which 80% or more of the occupants would be expected to find the environment thermally comfortable.[24] ASHRAE also recommends that RH be maintained between 30 and 60% RH. Excessive humidities can support the growth of microorganisms, some of which may be pathogenic or allergenic.

RESULTS

Initial Survey

Visual Inspections

Inspection of various HVAC units housed inside of the building envelope did not reveal environmental conditions supportive of fungal growth and, subsequently, obvious fungal contamination. The interiors of the inspected systems' working components appeared dry and clean. However, the insulation lining of the return air ducts located in each of the serviced classrooms exhibited considerable debris (i.e., dust agglomerated to the duct lining and trash deposited in the plenum). In contrast, the AHUs located beneath the D wing crawlspace (AHU 12 and 13) showed evidence of flooding into the outdoor air supply ducts. The flooding was evidenced by the residual water line in the duct interior and water remnants bound into the fibers of the exterior duct lining.

During the walk–through investigation, active fungal growth was observed in only a few locations inside the building. Significant growth was observed in the Home and Family classroom at the return air diffuser beneath the linoleum floor. Reports from the faculty indicated that the floor of this room was flooded recently due to a burst water pipe. Bubbles in the linoleum were observed by the NIOSH investigators, likely a direct result of water incursion. Fungal colonies were also observed on ceiling tiles that had been left in the attic space above some of the classrooms. These tiles had reportedly been removed earlier in the year as a result of water damage from a leaking roof, but some of the tiles still remained scattered along the top of the ductwork. Although the crawlspace underneath the D wing had a musty odor, there was no visible evidence of active fungal growth. However, occupants stated that the dirt floor had recently been raked. Active ground water incursion into the crawlspace was observed at various points in the foundation. In addition, a plastic sheet was observed below the dirt floor. This "vapor barrier" will result in the inability of water to drain out of the crawlspace. The pooling created by water intrusion into the crawlspace and the dirt floor make the likelihood of fungal growth at various locations within the crawlspace highly probable.

Bulk Sample Analysis

Fungal concentrations from the bulk material samples ranged from non–detectable (ND) to 6.4×10^6 colony forming units per gram of material (CFU/gm) and are summarized in Table 1. The predominant fungi identified included *Aspergillus versicolor*, *Chaetomium*, *Penicillium*, *Paecilomyces*, *Stachybotrys chartarum* (a.k.a. *S. atra*), *Rhodotorula*, *Sporobolomyces*, and unidentified yeasts. These genera have been implicated as allergens and *Aspergillus*, *Penicillium*, and *Stachybotrys chartarum* have been additionally noted as mycotoxin producers. *Chaetomium*, *Stachybotrys chartarum*, *Rhodotorula*, *Sporobolomyces*, and unidentified yeasts are characterized as hydrophilic (moisture–loving) fungi. Most of the bulk material samples revealed low concentrations of fungi which is not consistent with the existence of an active microbial reservoir. However, 7 of 20 bulk material samples resulted in high fungal concentrations and/or were identified with significant genera.

The wood floor sample collected from the Home and Family classroom had concentrations of *Aspergillus versicolor* and *Chaetomium* ranging to 1.4×10^6 and 1.8×10^6 CFU/gm, respectively. This is consistent with the sticky tape sample from the flooring that also revealed predominantly *Aspergillus* sp. and *Chaetomium*. Additionally, bulk material samples from the lining of the two return air duct plenums in the Home and Family Life classroom revealed *Aspergillus* sp. and *Chaetomium*, although in low concentrations. Bulk material samples collected from the exterior thermal insulation of the outdoor air intake of AHU 12 revealed high concentrations of Gram negative and Gram positive bacteria (3.3×10^6 and 2.4×10^7 CFU/gm, respectively) and concentrations of *Stachybotrys chartarum* ranging up to 7.7×10^5 CFU/gm. A bulk material sample collected from the interior duct lining of AHU 13 (before the filters) revealed the presence of a low concentration of *Stachybotrys chartarum* (769 CFU/gm) and, additionally, *Penicillium* ranging to 1.6×10^4 CFU/gm. Bulk material samples from two ceiling tiles found in the attic space above the school were cultured with fungal concentrations ranging up to 4.7×10^6 CFU/gm; the predominant genera identified included *Rhodotorula*, unidentified yeasts, *Penicillium*, and *Sporobolomyces*.

Bacterial concentrations from the bulk material samples ranged from ND to 1.6×10^7 CFU/gm. Gram negative bacteria were the major type detected and are normally found in association with large amounts of moisture. The highest bacterial concentrations were found in the floor sample from the Home and Family Life classroom, in both of the outdoor air intake exterior thermal insulation samples collected from AHU 12, and in the ceiling tile samples.

Microbiologic analysis of the nine debris and dirt samples indicated fungal levels ranging from 344 to 2.6×10^6 CFU/gm and bacterial concentrations ranging from 1.1×10^4 to $>5.4 \times 10^7$ CFU/gm (Table 2). The predominant fungal genera identified include

Acremonium, *Penicillium*, *Cladosporium*, and unidentified yeasts; the predominant bacterial type was Gram negative. Significant concentrations (greater than $1x10^6$ CFU/gm) of fungi or bacteria were found in the dirt samples collected in the D wing crawlspace (under Room 302 and under the AHU 12 outdoor air intake). Moderate concentrations (greater than $1x10^4$ CFU/gm) of fungi and bacteria were detected in all remaining samples (including the supply air diffuser debris in the Business classroom) except those collected from the water dampened areas of the crawlspace dirt and the pooled water in the AHU 12 exterior duct lining. However, the lack of fungal growth from the exception samples is not unusual as fungi do not tolerate low oxygen levels in water saturated environments. Although in low concentrations, *Aspergillus versicolor* and *Stachybotrys chartarum* (mycotoxin producers) were identified in the crawlspace dirt.

Comfort Indices

Carbon dioxide concentrations ranged from 300 to 1225 ppm in the 21 locations measured in the building. The mean concentration was 520 ppm. Two measurements, 1200 ppm in Room 104 and 1225 ppm in Room 100, exceeded the 1000 ppm recommended in the ASHRAE Standard 62–1989 as the upper limit for comfort (odor) reasons. The outdoor air CO_2 concentration was 300 ppm.

Temperatures in the building ranged from 68° to 76°F, with a mean of 71°F. Relative humidity ranged from 53% to 60%, with a mean of 56%. These measurements were within the comfort zone recommended in ASHRAE Standard 55–1982; however, the combination of temperature and RH might feel somewhat cool to those in summertime clothing.

Follow–up Survey

Visual Inspection

During the follow–up survey, some moisture incursion was noted in the D wing crawlspace, primarily through pipe and conduit entries through the foundation. During a period of rain during the survey, water drained into the below ground locations where the air intakes for AHUs 12 and 13 are located. It should be noted that these two AHUs were still not being operated, and ventilation to the D wing classrooms was supplied through open doors or windows.

Two additional areas identified during the initial survey as potential reservoirs for microbiological contamination appeared to have been eliminated. The ceiling tiles had been removed from the attic spaces above the classrooms, and the remaining tiles above the kitchen area had reportedly been inspected and no microbial growth was found. The linoleum, underlayment, and parts of the subfloor in the Home and Family classroom had been removed and replaced; however, some employee reports indicated that no containment procedures were used during this procedure. No additional microbial reservoirs were noted during this survey.

Culturable Air Sample Analysis

A graphical summary of the bioaerosol sampling results for fungi is presented in Figure 1. No significant differences existed in concentration or the predominant taxa between the different nutrient media (MEA and DG18) at each of the various sampling locations; therefore, the data collected using different nutrient media at each location was pooled. The geometric mean fungal concentration at various locations inside the building ranged from 99 colony forming units per cubic meter of air ([CFU/m^3] geometric standard deviation of 1.2) in the faculty lounge to >1492 CFU/m^3 (geometric standard deviation of 1.1) in the crawlspace. Due to overgrowth on the culture plates collected in the crawlspace, the concentration is an estimate that

assumes a CFU under every impaction hole. Outside of the building, the geometric mean fungal concentration was 365 CFU/m^3 (geometric standard deviation of 2.2). All of the indoor fungal concentrations were below the outdoor geometric mean level, however, the concentrations in four of the classrooms (i.e., Spanish, Business, Home and Family, and CAD) approached the outdoor concentration.

The taxonomic ranking (i.e., the ranking of the predominant genera according to frequency occurrence) of many of the indoor locations was dissimilar to the ranking observed outdoors. Specifically, a higher percentage of Penicillium sp. (indicated by the line with the square markers in Figure 1) was identified in the Computer Lab, Room 100, Spanish, Home and Family, CAD, and the faculty lounge. Outdoors, the percentage of *Penicillium* averaged 14%, whereas indoors, in the aforementioned locations the percentages ranged from 27% to 44%. *Aspergillus* sp. (indicated by the circles in Figure 1) were also identified in the Computer Lab, Room 100, Spanish, Home and Family, and CAD. The percentages of *Aspergillus* species identified were low by comparison; however, no *Aspergillus* species were identified outdoors.

The results of air sampling for total spores are shown in Table 3. The spore concentrations should only be used as qualitative indicators of spore levels at each location, due to problems encountered with the sampling method (i.e., sampling closed face) that may have resulted in an under–estimation of the total spore count. Like the culturable sampling results, greater relative numbers were observed for the samples collected outdoors compared with indoor sites. The most significant event observed from the spore samples was the identification of Stachybotrys spores from the crawlspace sample. Although the relative concentration is low, the finding is consistent with the bulk sample results which show the presence of culturable *Stachybotrys* in samples collected from the exterior duct lining of AHU 13 and in the soil within the crawlspace.

Comfort Indices

Carbon dioxide concentrations ranged from 425 ppm to 1150 ppm, with a mean concentration of 650 ppm in the 13 samples. Two measurements (Rooms 100 and 104) exceeded the 1000 ppm recommended in the ASHRAE Standard 62–1989 as the upper limit for comfort (odor) reasons. In both instances, classrooms were occupied by 20 or more students. The outdoor air CO_2 concentration was 350 ppm during the follow–up survey.

During the follow–up survey, temperatures in the building ranged from 67° to 71°F, with a mean of 69°F, and RH ranged from 53% to 59%, with a mean of 56%. These measurements were within the comfort zone recommended in ASHRAE Standard 55–1982; however; the combination of temperature and RH might feel somewhat cool to those in summertime clothing.

DISCUSSION AND CONCLUSIONS

The growth and survival of microorganisms in environmental reservoirs requires (a) a suitable nutrient source; (b) adequate available water; and (c) an appropriate temperature. These factors are all determined by the localized environment and when combined with high porosity materials, can provide optimum conditions for microorganisms to grow. In the crawlspace under the D wing, these factors were all present; i.e., water intrusion from snow melt and rain storms (present in the duct lining and in the dirt floor), organic material in the dirt floor, and cool temperatures (that result in increased humidity). Microbial contamination was confirmed by bulk sample analysis in select locations of the dirt floor and from exterior lining in the outdoor air intakes of the AHUs. Some of the fungal species identified included the mycotoxin producers *Aspergillus versicolor* and *Stachybotrys chartarum*. The culturable air sample results showed the existence of large concentrations of fungal species (as evidenced by the overgrowth for short sampling times) which

included *Aspergillus* and *Penicillium* species. These large concentrations demonstrate the active dissemination of fungal reservoirs in the crawlspace. The upstream side of the fan in AHUs operate under a negative static pressure. Placement of the AHUs in the crawlspace will inevitably draw air and contaminants from the crawlspace (and contaminants in the exterior duct insulation) into the system to be disseminated to the occupied spaces. Therefore, a potential pathway exists for the dissemination of microbial contaminants into the air spaces supplied by these AHUs. This clearly indicates that this is not a suitable location for the AHUs. It would also indicate a need to keep the building areas under a positive pressure, with respect to this space, to prevent entrainment of microbial contamination into the classrooms.

Growth conditions were also present in the Home and Family classroom as evidenced by the "bubbling" of the linoleum floor noted during the initial survey visit, and subsequent growth of *Aspergillus versicolor* and *Chaetomium* in the wood underlayment. However, the microbial contamination in this classroom should be considered as localized given that a substantial pathway to the occupied areas is not present except at the flooring seams. However, bulk material sample analysis of the return air plenum revealed evidence of small numbers of *Aspergillus versicolor* and *Chaetomium* spores and/or hyphae in the interior thermal insulation. This would seemingly indicate that some dissemination of the microbial contaminants was occurring from the wood underlayment. Although the linoleum floor had been replaced by the September 1997 follow–up visit, the observed fungal concentrations (as shown by the culturable air sample results) were among the highest observed in the school. In addition, the contribution by *Penicillium* species to the total fungal load was the highest percentage (approximately 44%) compared to all other sample locations. These levels may not necessarily be indicative of current reservoirs, but rather an indication of inappropriate (or non–existent) containment during (and inadequate cleaning after) the removal of the old linoleum floor and the installation of the new floor. A more thorough cleaning of this area appears warranted.

The bulk samples and visual observation did not reveal the existence of substantial microbial reservoirs in the other areas of the school at the time of the site visits. Furthermore, no evidence of ongoing moisture incursion or moist conditions was noted, which is needed for ongoing growth of microbial contaminants. Since separate air handling systems supply the different areas of the school, no common dissemination pathway would be expected to exist from those areas of the school where microbial reservoirs were noted. This would tend to be supported by the culturable air and spore sample results which indicated all of the indoor fungal concentrations were below the outdoor level. The dissimilarities found in the taxonomic ranking of many of the indoor locations is somewhat more difficult to interpret. The higher levels of *Penicillium* sp. identified in some locations may be indicative of a small residual from past contamination (such as during the remodeling or incidents of flooding) or dissemination (from the AHUs in the D wing crawlspace) with ineffective cleanup. However, this conclusion is speculative based on the inherent limitations of this type of data. Due to the high level of concern, and the fact that a potential for microbial contamination existed from the previously described events, it would seem prudent to make all reasonable efforts to thoroughly clean these areas to remove any doubt of residual contamination.

RECOMMENDATIONS

D Wing Crawlspace

The crawlspace should not be used as a location for AHUs and/or associated negative pressure ductwork. Unit ventilators located in the classrooms or centrally located AHUs should be used to provide air to these building. The ventilation system(s) in the D wing should be operated in a manner so as to keep the occupied building spaces at a positive pressure with respect to the crawlspace. The use of an

additional exhaust fan(s) in the crawlspace may help insure this pressure differential.

Building Cleaning

Visible or suspected microbial contamination requires remediation efforts including the removal of the contaminated material and/or clean–up with a high efficiency particulate air filter (HEPA) vacuum and decontamination with an effective chemical agent (i.e., 5 to 10% solution of chlorine bleach). Remediation will result in the disruption of microbiological reservoirs. The airborne dissemination of these bioaerosols can pose a significant exposure concern for the remediation workers. Additionally, these aerosols can be spread to uncontaminated areas of a building, increasing the hazard for the remaining occupants and adding to the difficulty of clean–up. Therefore, it is important that all remediation activities be conducted with an awareness of the potential bioaerosol exposures and with minimal disturbance of contaminated materials. Specifically, controls must be instituted that protect both the *worker* and the *adjacent environment*.

Remediation workers should use personal protective equipment (PPE) appropriate for the hazards to which they may be exposed. Such decisions require *a priori* awareness of potentially hazardous agents, significant exposure routes (e.g., inhalation, dermal contact, or ingestion), and possible concentrations of the biological materials. Remediation work on small, localized patches of mold growth on ceilings or walls should be conducted with appropriate respirators (i.e., a disposable N–95 NIOSH–approved respirator with a facepiece that fits tightly, ensuring that contaminants do not enter through leaks between the respirator and a wearer's face), eye protection, and gloves. However, situations involving gross contamination with microorganisms that pose potentially significant health outcomes (e.g., infectious or toxigenic fungi), may require a higher level of PPE due to the concentrations of the microbial agents and their disease potential (e.g., full–face, powered air–purifying respirators, disposable protective clothing with hoods, gloves, and disposable shoe coverings). For respirator use, OSHA requires a respiratory protection program that includes the following components: written standard operating procedures, user instruction and training, cleaning and disinfection, storage, inspection, surveillance of work area conditions, evaluation of respirator protection program, medical review, and use of certified respirators.[42]

Given the level of disruption that may occur during microbiological remediation work, engineering controls applied at the source should be the primary control measure. Remediation activities should be conducted in a manner that minimizes the disturbance of microbiological reservoirs. However, as the extent of the microbial contamination becomes larger, reservoir dissemination becomes unavoidable due to the activities of surrounding building material removal. Under these conditions, isolation barriers are required to contain airborne spores and other biological matter. Barriers alone disrupt the pathways between remediation zones and adjacent environments, but disseminated aerosols almost invariably find breaks in any barrier system. Therefore, negative pressure relative to adjacent areas is induced in the remediation zone to ensure containment. It is critical that the exhausted air streams be appropriately filtered (i.e., HEPA filters) to guard against the re–entry of microbially contaminated air back into the zone of remediation and/or to other areas that are considered uncontaminated. Specific control guidelines have been recommended for the remediation of toxigenic fungi from contaminated materials.[43]

Water Incursion Incidents

Any episodes of water incursion should be dealt with promptly. Water should be removed immediately from porous, water damaged furnishings, carpets, and construction materials. Heat fans should be used to dry carpets and other applicable surfaces within 24 hours. Steam or other water–based cleaning methods which add moisture to the environment must be used with extreme care. Any soft materials that become wet with sewage contaminated water should be promptly discarded. A written program

for dealing with these incidents, proper training of personnel, and the ready availability of the necessary equipment would help reduce the likelihood of future problems from events of this nature.

Operation and Maintenance of HVAC Systems

Continuing attention should be given to ensuring the comfort of the building occupants through regulation of temperature (in consideration of the RH) and the supply of adequate amounts of outside air to the occupied spaces. Written logs of unit inspections and filter changes should be maintained. Return air ducts in the classrooms should be regularly inspected and dirt and debris removed as needed.

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Table 1. Microbiological Results of Bulk Samples

Sample Location	Fungi (CMA)		Fungi (MEA)		Bacteria (TSA)	
	(CFU/gm)	Taxa Rank	(CFU/gm)	Taxa Rank	(CFU/gm)	Taxa Rank
B1 (Home and Family – under linoluem)	$4.1x10^6$	Chae>Pen>A. ver	$3.4x10^6$	A. ver>Pen>Chae	$1.6x10^7$	G neg
B2 (Room 301 – return duct insulation)	ND		ND		752	unidentified
B3 (Home and Family – return duct insulation 1)	$1.5x10^3$	Chae>Pen>A. ver= Pen= Clad	$3.5x10^3$	Chae	ND	
B4 (Home and Family – return duct insulation 2)	$4.0x10^3$	Pen>Clad	$6.5x10^3$	Pen>Cha=Y	806	unidentified
B5 (Room 300 – return duct insulation)	$1.7x10^3$	Pen=Pae	$1.7x10^3$	Pen>stf>Pae	$1.7x10^3$	unidentified
B6 (Room 103 – unit ventilator insulation)	813	Y	ND		ND	
B7 (Room 200 – unit ventilator insulation)	$4.0x10^3$	Pen>Clad>Chae	16,200	Pen>>Clad	$1.0x10^3$	unidentified
B8 (AHU 12 – exterior insulation below fan)	$9.5x10^3$	Y=Clad>Chae	$4.8x10^3$	Pen>Epi=Clad=Chae	$1.8x10^4$	G neg>Bac
B9 (AHU 12 – outside air intake exterior insulation 1)	$5.8x10^5$	Sta>>Y=Dor	$8.7x10^5$	Sta>>Pho>A. Fum= stf=Y	$3.3x10^6$	G neg
B10 (AHU 12 – outside air intake exterior insulation 2)	$2.1x10^3$	Y=Aur>Tri	592	Pen=Tri	$>2.4x10^7$	unidentified
B11 (AHU 12 – interior insulation)	$7.3x10^3$	Clad>Pen>Asp	$7.3x10^3$	stf>Clad=Y>Pen	$6.1x10^4$	G neg>Bac
B12 (AHU 12 – interior insulation 1)	$2.1x10^4$	Pen>>Y>Clad>stf=Sta	$1.8x10^4$	Pen>>A. nig=Clad>Ulo	$3.0x10^4$	G neg>Bac>Mic= Rho
B13 (AHU 12 – interior insulation 2)	$4.7x10^3$	Pen>Clad=stf	$3.7x10^3$	Pen	$1.7x10^4$	G neg
B14 (Room 100 – unit ventilator insulation)	ND		$3.3x10^3$	Clad=Pen=Pith=Y	$3.3x10^3$	unidentified
B15 (AHU 1 – interior insulation 1)	$4.2x10^3$	Pen>stf	$5.8x10^3$	Pen>Clad	$4.2x10^3$	unidentified
B16 (AHU 1 – interior insulation 2)	ND		855	Pen	$7.7x10^3$	G neg
B17 (Room 100 – carpet)	909	Pen	909	Pen=Acr	455	unidentified
B18 (ASB room – carpet)	$8.7x10^3$	Pen>Y	$8.7x10^3$	Pen>Y	$3.9x10^4$	G neg
B19 (Ceiling tile 1)	$3.4x10^6$	Y>Rho>>Spo>Aur= Chry=Pen	$3.2x10^6$	Y>Rho>>Spo>Aur=Pen	$2.3x10^5$	G neg>Bac
B20 (Ceiling tile 2)	$6.4x10^6$	Y>>Spo>Pen>Chry>Rho	$3.6x10^6$	Y>>Spo>Pen>Chry=Rho	$1.8x10^6$	G neg>Bac

Acr – *Acremonium*
A ver – *Aspergillus versicolor*
A nig – *Aspergillus niger*
Aur – *Aureobasidium pullulans*
Chae – *Chaetomium*
Chry – *Chrysoprorium*
Clad – *Cladosporium*
Dora – *Doratomyces*
Epi – *Epicoccum nigrum*
Pae – *Paecilolmyce3s variotii*
Pen – *Penicillium*
Pho – *Phoma*
Pith – *Pithomyces*
Rho – *Rhodotorula*
Spo – *Sporobolomyces*
Sta – *Stachybotrys chartarum*
stf – sterile fungi
Tri – *Trichoderma*
Ulo – *Ulocladium charatarum*
Y – unidentified yeast
Bac – *Bacillus*
G neg – Gram negative
Mic – *Micrococcus*
Rho – *Rhodococcus*

Table 2. Microbiological Results of Bulk Samples (debris and dirt)

Sample Location	Fungi (CMA)		Fungi (MEA)		Bacteria (TSA)	
	(CFU/gm)	Taxa Rank	(CFU/gm)	Taxa Rank	(CFU/gm)	Taxa Rank
V1 (crawlspace soil under Room 302)	$2.6x10^6$	Acr >Pen>>A. ver=Clad	$2.1x10^6$	Pen>Acr>>Tri>Clad	$>5.4x10^7$	G neg
V2 (crawlspace soil 1 under AHU 12)	$4.2x10^3$	Pen>Mon=Pho=Sta=Tri	$4.2x10^3$	Glio>Pen>A. ver=Tri	$7.4x10^4$	G neg>>Bac>Act
V3 (crawlspace soil under AHU 13)	$3.7x10^3$	Pen>stf>Tri	$1.9x10^4$	Sta>Pen=Y>stf>Pho=Pith	$1.8x10^5$	G neg=Bac
V4 (crawlspace soil 2 under AHU 12)	$3.7x10^4$	Clad>Acr>Ver=Trit>stf	$2.4x10^4$	Clad>Acr>Trit>Glio=Pho=Pith=Ver	$4.4x10^6$	G neg>>Mic>Rho>Bac
V5 (crawlspace soil at water incursion point 1)	344	Tri	344	Tri	$4.7x10^4$	Bac
V6 (crawlspace soil at water incursion point 2)	$1.1x10^3$	Pen=Tri=Muc	$2.2x10^3$	Y>Tri=Muc	$3.7x10^4$	Bac
V7 (Home and Family – debris from diffuser)	$5.5x10^4$	Pen>>Chae>stf	$4.1x10^4$	Pen>>stf>Clad=Epi=Y	$2.3x10^5$	Bac
V8 (Business – debris from diffuser)	$9.2x10^4$	Y>Pen>Clad>Curv	$1.4x10^5$	Y>Aur=Clad=Pen>Alt=Rho	$1.8x10^5$	G neg>Bac
V9 (AHU 12 – water in exterior insulation)	440	Pho>Myc=Acr	220	Pen=Tri	$1.1x10^4$	Bac

Acr – *Acremonium*
A ver – *Aspergillus versicolor*
Alt – *Alternaria*
Aur – *Aureobasidium pullulans*
Chae – *Chaetomium*
Cur – *Curvularia*
Clad – *Cladosporium*
Glio – *Gliomastix*
Epi – *Epicoccum nigrum*
Mon – *Monodictys*
Muc – *Mucor*
Myc – *Mycotypha*
Pen – *Penicillium*
Pho – *Phoma*
Pith – *Pithomyces*
Rho – *Rhodotorula*
Spo – *Sporobolomyces*
Sta – *Stachybotrys chartarum*
stf – sterile fungi
Tri – *Trichoderma*
Trit – *Tritirachium*
Ver – *Verticillium*
Y – unidentified yeast
Bac – *Bacillus*
G neg – Gram negative
Mic – *Micrococcus*
Rho – *Rhodococcus*

Table 3. Spore Air Sampling Results

Sample Location	Date	Concentration (spores/m^3)	Taxonomic Rank
Computer Lab	9/15/97	20	Clad>Asp/Pen
	9/16/97	10	Asp/Pen=hyph
Room 100	9/15/97	30	Clad>unk>Alt
Spanish	9/16/97	10	unk
Business	9/15/97	40	Asp/Pen>Clad=Epi>Alt
	9/16/97	10	Clad=unk
Home and Family	9/15/97	10	Clad=unk
	9/16/97	20	unk>hyph
CAD	9/15/97	10	Clad>unk
Faculty lounge	9/16/97	30	Clad>unk
Outdoors	9/15/97	220	Clad>>asc>unk>bas>hyph>Sco>Asp/Pen
	9/16/97	170	Clad>>unk>Asp/Pen>asc>bas
Crawlspace	9/16/97	40	unk>Stachy

asc = ascospores
Alt = *Alternaria*
Asp/Pen = *Aspergillus*/*Penicillium* like
bas = basidiospores
Clad = Cladosporium
Epi = *Epicoccum*
hyph = hyphal fragments
Sco = *Scopulariopsis*
Stachy = *Stachybotrys*
unk = unknown

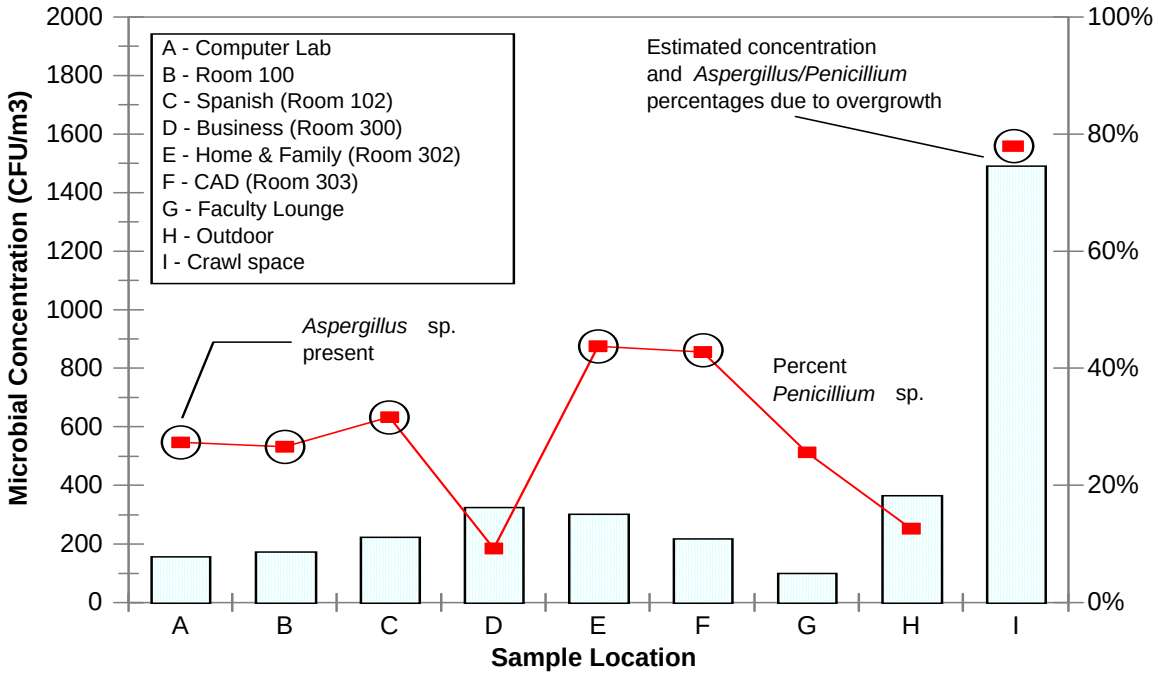

Figure 1. Culturable Air Sample Results for Fungi

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