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Emission Exposure Model for the Transport of Toxic Mold

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Key Words
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Abstract
Biocontaminants such as mold spores are capable of being released into the indoor air from the site of growth and being transported in a viable or non-viable form. Exposure to toxic mold and the mycotoxins contained in the spore and vegetative body have been shown to produce adverse health effects following inhalation, ingestion and dermal contact. This paper presents the results of a study of the release of *Stachybotrys chartarum* spores from contaminated gypsum wallboard and of tests on the effects of environmental conditions on the release of viable and non-viable spores and fragments. The findings of *S. chartarum* spore emissions with low air velocity flow conditions were found to be directly proportional to airflow and indirectly proportional to relative humidity. These emission findings corroborate previous observations involving *Penicillium* and *Aspergillus*. The viability of *S. chartarum* spore emissions is also discussed with respect to culturable and commonly used field measurement techniques.

Introduction

Mold contamination in the indoor environment is recognised as a major health concern [1]. Exposure to airborne mold contaminants or their metabolites can induce responses including irritation, allergy, and infection and acute reactions such as vomiting, diarrhoea, haemorrhage, convulsions and, in some cases, death [1–5]. The application of effective engineering controls within the building is essential to prevent biological pollution in the indoor environment.

The study of particle emissions from areas of mold contamination is important and necessary to the understanding of transport and deposition of biologically based contaminants in the indoor environment. At least 90% of our exposure comes within the indoor environment [6]. It is widely accepted in the IAQ research community that biocontaminants are one of the important indoor air pollutants. Airborne mold spores, their metabolites, and fragments of mycelia make up a portion of the particulate matter which is biological. Salvaggio and Aukrust [7] found *Cladosporium* spores in ambient air at a concentration 1000 times the concentration of pollen grains. Aerometric sampling devices have collected spores from 20,000 to 40,000 species of fungi. Of these, four major groups have been identified as being potential allergens: Phycomycetes, Ascomycetes, Basidiomycetes and Deuteromycetes [7].

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Mold based particles have been identified in various size fractions of airborne particulate matter [8]; 2.5–3.0 μm for *Aspergillus fumigatus*, 3.5–5.0 μm for *A. niger*, 3.0–4.5 μm for *Penicillium brevicompactum*, 7–17 μm by 5–8 μm for *Cladosporium macrocarpum*, 15.0–25 μm for *Epiccocum nigrum*, and 2.8–3.2 μm for *Trichoderma harizanum* [9]. These fragments may be toxic and allergenic depending upon the specific organism or organism component.

The adverse health effects associated with exposure to indoor mold contamination has been regarded chiefly as due to its properties as an allergen and, occasionally, as a pathogen [10]. Many varieties of mold produce metabolites which are toxins of low molecular weight (mycotoxins) and which have economic as well as human and animal health importance associated with contamination of food and feed. Mycotoxins concentrate in mold spores [11] and spore fragments, which can easily become airborne and mobile in the adjacent air-stream [12]. Mycotoxin containing spores are hazardous and known to affect alveolar macrophage function following exposure. *Stachybotrys chartarum* and several toxigenic species of *Penicillium* and *Aspergillus* have been indicated as being in this group of hazardous mold [10].

This paper describes the results of research to determine the factors controlling the release of *S. chartarum* spores from a site of active growth and describes tests on how a changing environment affects spore release and spore viability. Numerous "sick buildings" have been reported to be contaminated with *S. chartarum*. However, the study of environmental conditions permitting the growth and distribution of mold spores has been limited. Problems inherent with air sample collection in the field and with identification, have hampered the understanding of *S. chartarum* exposure. To address this issue, data recorded on released spores and spore fragments concentrations was used with the RISK exposure model to generate an exposure model for a typical school environment.

Materials and Methods

The Dynamic Microbial Test Chamber (DMTC) [13], a room-sized test containment facility, was used to conduct the experiments. The DMTC was designed and constructed to perform biological growth experiments under controlled environmental conditions that affect spore growth, emissions and distribution. The chamber has an inside volume of $2.44\,m^3$ and is made with stainless steel walls, floor, and ceiling with acrylic windows set in the walls. Controlled air temperatures of 18–32°C and relative humidity (RH) of 55–95% could be provided through an external air handler unit (AHU) with a re-circulating airflow rate of $1.4–4.8\,m^3 \cdot min^{-1}$. Conditioned air is circulated through a high-efficiency particulate arresting (HEPA) air filter for complete removal of particles larger than 1 μm. Eight room-wall simulators (RWSs) were vertically mounted next to a window inside the DMTC, in which a pure culture of *S. chartarum* was grown. Figure 1 is an artist's rendition of the DMTC, including three of the eight RWS with attached external sample collection devices.

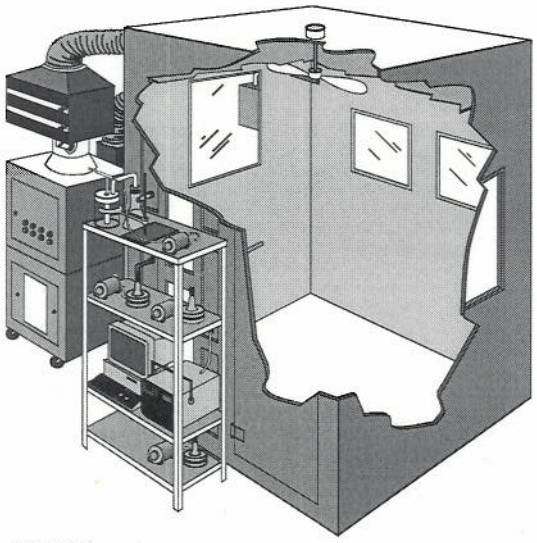

Fig. 1. Dynamic microbial test chamber (DMTC).

The RWSs (17.1 cm by 17.1 cm square section by 161.7 cm long duct section) are constructed of 16 gauge stainless steel. The front cover is made from glass to permit visual monitoring of growth on the test material. The top inlet and bottom discharge ends of the RWS transition to 2.5 cm diameter tubes. Each RWS was connected to a pressurized plenum fed by a blower drawing HEPA filtered and conditioned air from the DMTC. A single piece of gypsum board 107 × 42 cm (4494 cm^2) was scored length-wise to permit it to be folded into a three-sided trough that fitted into and formed the interior walls of the RWS. Air from the plenum entered each RWS through the inlet transition, flowed vertically down along the glass and three-sided gypsum board wall, and discharged through the outlet transition. Bioaerosol samples were drawn from the RWS discharge air through the bottom end into the tubing which penetrated the DMTC wall and into the sampling equipment.

The gypsum board pieces were loaded into the RWS and the entire assembly autoclaved. Each 4494 cm^2 gypsum board piece was wetted with 200 mL of sterile water three times for a total of 600 mL. After each wetting, the water was allowed to soak into the gypsum board, usually over a period of seconds. Preliminary experiments measuring moisture showed that the water wicked throughout the entire piece and that a uniform distribution of water over all surfaces was easily achieved. The goal was to simulate a catastrophic wetting of the material and monitor mold growth in colony forming units (CFU). The gypsum board was then inoculated with 12.9 mL of 10^6 CFU · mL^{-1} *S. chartarum* manually pipetted in 15 µL spots uniformly distributed across the surface of the gypsum board.

The RWSs were sealed and mounted vertically on a rack within the DMTC and growth was allowed to proceed under static conditions for at least one month until relatively heavy growth was visible on the gypsum board surface. Air was then allowed to flow in at the top and out at the bottom at a vertical velocity of either $< 10\,cm \cdot s^{-1}$ ($117.0\,L \cdot min^{-1}$) or $35\,cm \cdot s^{-1}$ ($409.5\,L \cdot min^{-1}$), on the order of velocities encountered in the conditioned indoor space [14]. This flow is designed to closely resemble real-world conditions and near wall air velocities.

At the initiation of the emission rate measurements, each RWS was unsealed and airflow at a temperature of 23.5°C and RH specified in the experiment (95, 85, 75, or 65%) was begun. Isokinetic air samples were collected using the Mattson–Garvin slit to the agar sampler for culturable fungi and Air-O-Cell cassettes (Zefon Analytical Accessories, Saint Petersburg, FL) for total spore counts.

The Mattson–Garvin draws air at $28.3\,L \cdot min^{-1}$ through a metal inlet with a 0.015-cm slit, allowing the impaction of airborne organisms on the surface of a rotating 150-mm agar plate. The sampler plates were incubated at room temperature. CFUs were counted shortly after visible growth was first noted and again as moderate growth became apparent.

Air-O-Cell cassettes contain a glass slide coated with a sticky impaction medium. The base of the cassette was connected to a pump using flexible tubing, and air was drawn onto the impaction surface at $28.3\,L \cdot min^{-1}$ through a slit in the top of the cassette. Total airborne spores were quantified by opening the Air-O-Cell and removing the internal glass slide containing the impaction medium. The slide was placed onto a microscope slide, stained with lacto-glycerol, counted microscopically to record the total airborne spores (viable and non-viable).

Calculation of Emission Rates

To calculate the emission rates of culturable organisms, the CFUs on the sampler plates from the Mattson–Garvin were enumerated, and the CFU · min^{-1} were determined. For total spores, the spores · min^{-1} from the Air-O-Cell cassettes were calculated. Both values were adjusted for the total flow rate and divided by the area of the emitting gypsum board surface.

Results and Discussion

Published experiments show that the spore emission rates for *Penicillium chrysogenum* and *Aspergillus versicolor* were directly related to airflow and indirectly related to relative humidity [15]. In Table 1, four separate RH levels (95, 85, 75 and 65%) of conditioned input air and two velocities (< 10 and $35\,cm \cdot s^{-1}$) were used to test emission rates. Emissions increased as the air velocity increased across the contaminated surface. The published experiments also found that as the RH was lowered, the

Table 1. CFU · m^{-2} · min^{-1} emitted at specified airflow and relative humidity

Airflow ($cm \cdot s^{-1}$)	Relative Humidity (%)			
	95	85	75	65
< 10.0	0.0	0.4	25.0	50.0
35.0	4.0	29.0	65.0	200.0

emission rate increased for both organisms [15]. These relationships were investigated to determine whether the spore emission rate for *S. chartarum* would follow a similar pattern.

The published work with *P. chrysogenum* and *A. versicolor* was performed on contaminated fiberglass duct liner under horizontal duct flow conditions and at air velocities inappropriate for wall air flow. The experiments this project conducted were on *S. chartarum* growing on selected gypsum wallboard and at lower vertical air velocities of 35 and $10\,cm \cdot s^{-1}$, which more closely approximates wall airflow [15].

Emission rates of culturable spores ($CFU \cdot m^{-2} \cdot min^{-1}$) from the first hour of airflow taken from a series of experiments at four levels of RH (from 65 to 95%) and two rates of air velocity (< 10 and $35\,cm \cdot s^{-1}$) are as shown in Table 1. These emission rates confirm that *S. chartarum* spore emissions increase with increased airflow and as the humidity is lowered.

The second set of experiments was designed to quantify emissions rates of four RWS, at 65% RH over an extended period (see Figure 2). The period was defined as that time during which measurements remained above the detection limit ($< 5\,CFU \cdot m^{-2} \cdot h^{-1}$). Surface concentrations of spores on the gypsum wallboard in the four RWS were essentially the same, ranging from 5×10^4 to 5×10^5 CFU/$10^2\,cm^2$. However, the age of the growth on the RWS 1, 2 and 3 was 2 months old when the emission experiments were initiated. RWS 4 was 3 months old and exhibited a higher sustained rate of emissions. Figure 2 displays spore emissions from RWS 1 through 3 as starting at levels between 500 and $1000\,CFU \cdot m^{-2} \cdot h^{-1}$ and tapering off by day 2 or 3. RWS 4 started at the same initial level as RWS 1, 2 and 3 but continued to emit that same level of spores for the entire first week before the level started to decrease.

The next set of experiments were designed to investigate the emission of spores at an RH below 65% and an air velocity of $35\,cm \cdot s^{-1}$. The RH was allowed to range from 25 to 64%, the normal range of room humidity in controlled environments. Figure 3 shows the hourly emission rates of both culturable CFUs and total

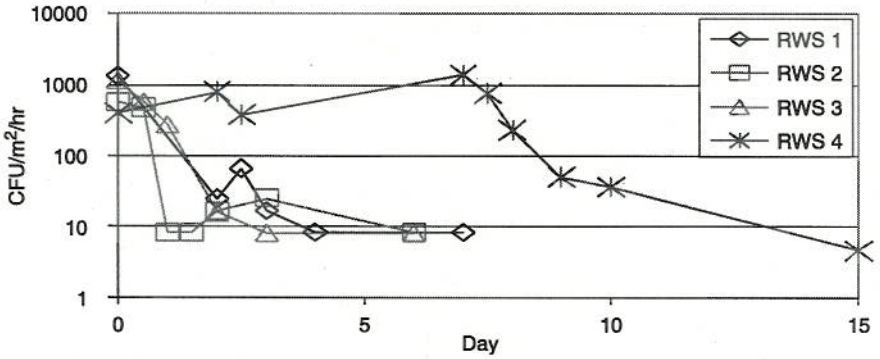

Fig. 2. S. chartarum CFU emissions from four RWS at 65% RH and $10\,cm \cdot s^{-1}$ airflow.

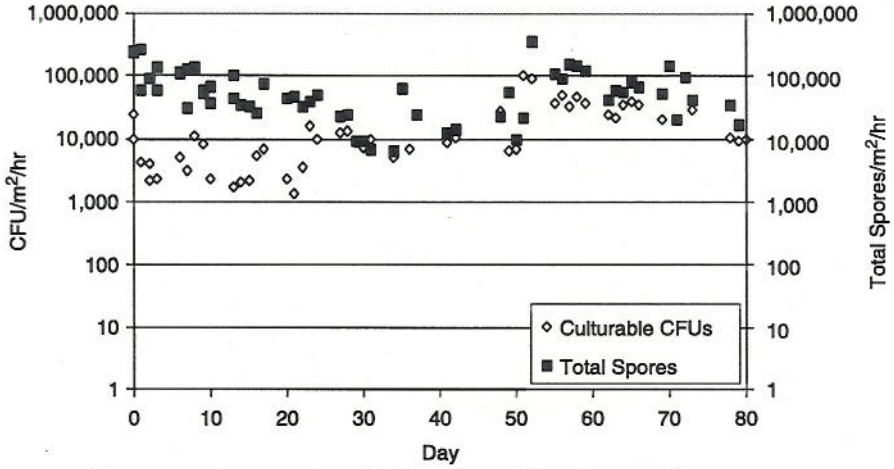

Fig. 3. S. chartarum spore emissions at RH between 25 and 64% at low air flow ($35\,cm \cdot s^{-1}$).

spores $\cdot m^{-2}$ from contaminated gypsum board from one RWS (3-months old). Culturable CFUs, as designated by the diamonds, are plotted on the left y-axis. Total spores are plotted on the right y-axis with squares. Figure 3 shows a sustained high level of spore emissions, continuing for the 80-day period.

Figure 3 indicates that for the first 30 days, the CFUs appeared to be about 10% of the number of total spores. After 30 days, the levels of culturable CFUs and total spores approach being equal. This ratio is depicted in Figure 4, showing the percentage of culturable CFUs to total spores over the nearly 3-month experiment. Figure 4 shows that, for the first 30 days, the culturable CFUs were approximately 10% of the total spores. For the next 30 days (days 30–60), the percentage of culturable spores increased notably compared to the first 30 days. For the final 25 days, the percentage of culturable CFUs was lower than the second 30 days, but higher than the first 30 days.

Use of an IAQ Model to Predict the Impact on IAQ

To better understand the implications of the emission rates measured in these experiments, an IAQ model was used to estimate concentration and potential exposure, as has been done with other indoor air contaminants. The goal was to estimate the potential indoor concentration levels using a typical emission rate from these experiments and reasonable ventilation parameters. The model used was RISK IAQ Model for Windows, which is a completely mixed room model incorporating source/sink behaviour that can generate concentration and exposure estimates as functions of time [16]. The ventilation flows and pollutant emission rates can be set as desired for each modelled room.

For this study, a school was modelled having 10 rooms distributed over a floor area of 371.6 m^2 with 2.44 m ceilings and served by one heating, ventilating, and air-conditioning system (HVAC). The total HVAC airflow to the area was 56.6 $m^3 \cdot min^{-1}$ (2000 cfm). The school was assigned three separate HVAC scenarios: (1) source with the HVAC always on (Figure 5) and no outdoor air; (2) source with HVAC on from 08.00 to 17.00 h (Figure 6), 9-h duty cycle, and no outdoor air; and (3) the same 9-h duty cycle as scenario 2 with the addition of an outdoor air intake level of 5% of the re-circulating airflow to the HVAC system as is the case in a typical school (Figure 7). The source of emissions was calculated from an area of mold contamination of 9.29 m^2 (100 ft^2), selected as representative of a modest area of contamination. Actual areas of mold contamination could be significantly different.

Concentration profiles were run, and the exposure levels were calculated for an average person (breathing at 0.83 $m^3 \cdot h^{-1}$ and, for simplicity, staying in the room 24 h $\cdot$ day^{-1}) for all scenarios. Various surfaces within a building, as well as components of a HVAC system may support microbial growth. The area of contamination was located with the school building which was served by the HVAC system as defined by each of the three scenarios modelled, and the results are shown in Figures 5, 6 and 7. The levels of Total Spore Emissions and the culturable CFUs for *S. chartarum* were modelled in these experiments based on emission data taken from Table 1 and Figure 2.

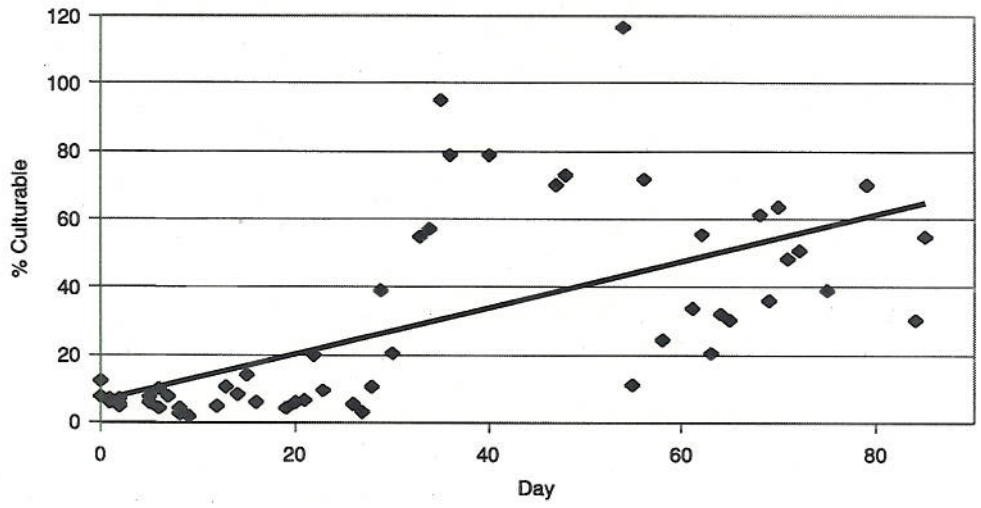

Fig. 4. Percent of the total spores that are culturable CFUs.

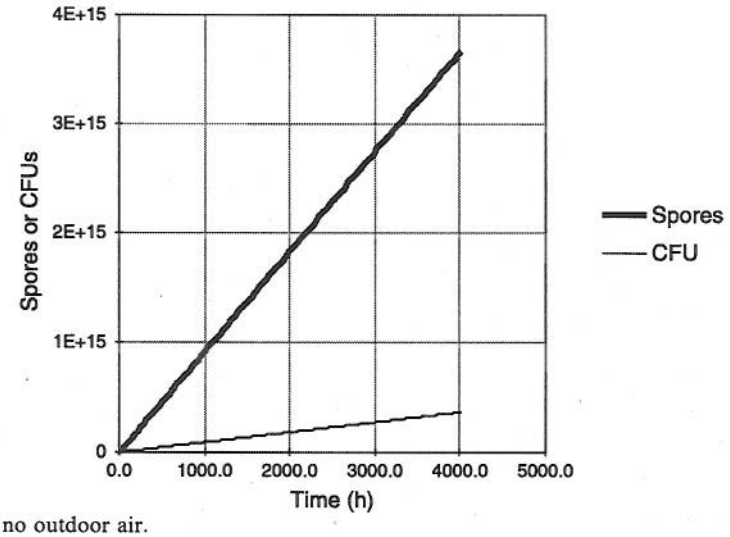

Fig. 5. HVAC always on and no outdoor air.

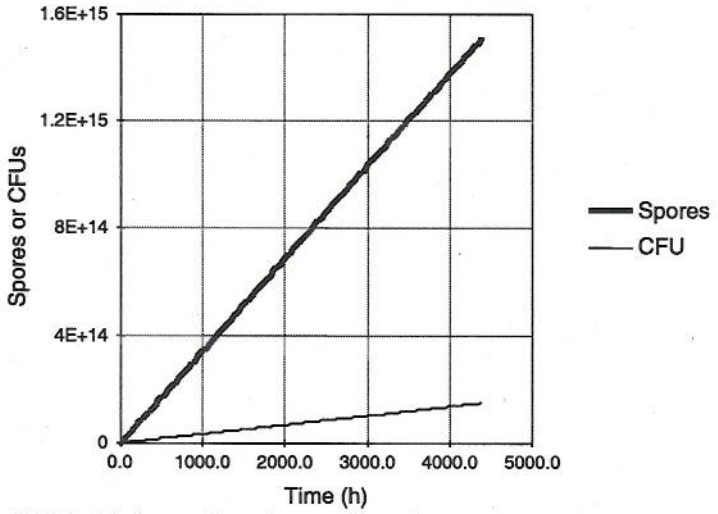

Fig. 6. HVAC on from 08.00 to 17.00 h, 9-h duty cycle, and no outdoor air.

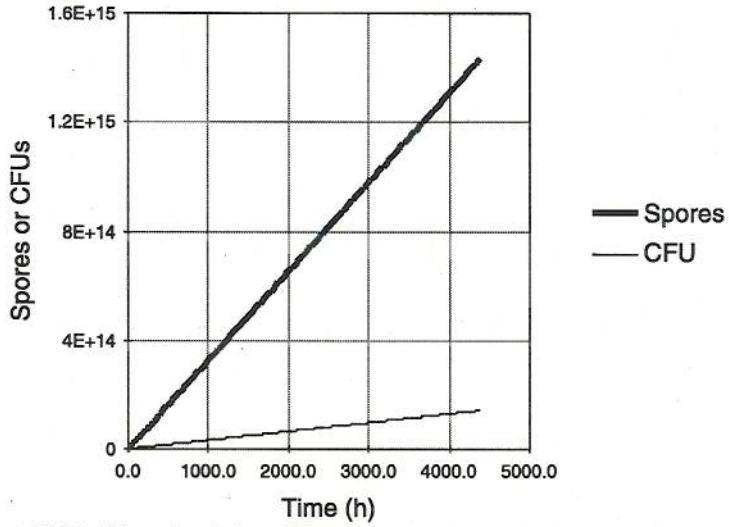

Fig. 7. HVAC on from 08.00 to 17.00 h, 5% re-circulating airflow.

Figure 5 depicts the modelled scenario of the HVAC always on without any outdoor air contribution to the recycle. In this figure spore levels approach 3.7×10^{15} total spores and 0.4×10^{15} CFUs after the system had been running constantly for 4000 h. These levels can be compared to the results of 1.4×10^{15} total spores and 1.4×10^{14} CFUs shown in Figure 6 (HVAC on from 08.00 to 17.00 h, a 9-h duty cycle, without outdoor air) or the results of 1.3×10^{15} total spores and 1.5×10^{14} CFUs shown in Figure 7 (HVAC on from 08.00 to 17.00 h and 5% airflow of recycle is contributed from outdoor air). From this evaluation, we can conclude that running the HVAC system constantly will result in the maximum transport of spores and the greatest concentration of both total spores and CFUs and, in comparison, is the worst scenario. A 9-h HVAC duty cycle, which is 37.5% of the time, has been calculated to produce a proportional decrease in total spores and CFUs.

Figures 8 and 9 indicate that an air filter which removes 40% of the particulate matter from the air stream can achieve a significant decrease in total spores and CFUs. The model specified a building dominated by the HVAC system and having a building air exchange rate of 3.8 ach. The inclusion of outdoor air can add a minor additional improvement, but not as significant as air filtration. This indicates that air filtration is able to reduce dramatically spore concentrations (Figures 8 and 9) from unacceptably high levels to background levels. It should be

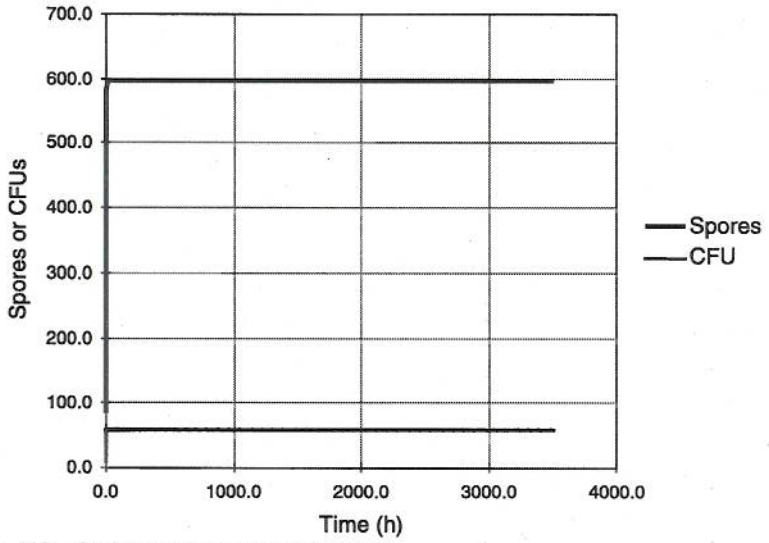

Fig. 8. HVAC on, 10% ODA, 40% air cleaner [Author explain ODA].

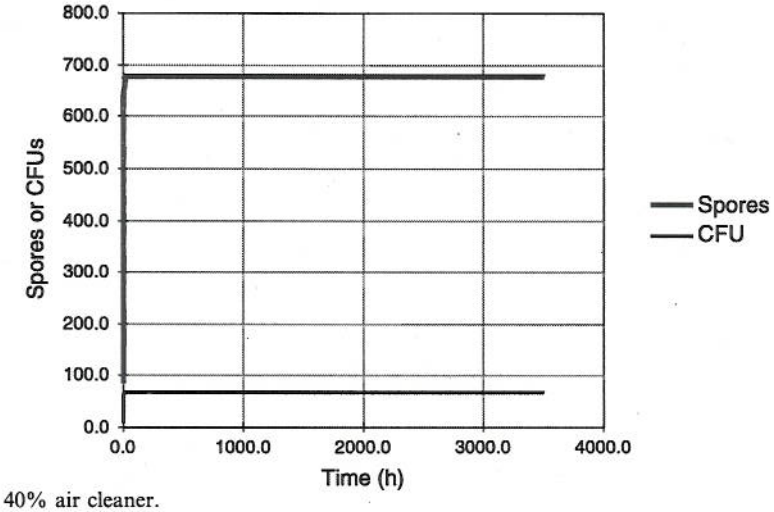

Fig. 9. HVAC on, no ODA, 40% air cleaner.

stressed that the model treats the entire area of the school as a well-mixed reactor, having equal concentrations regardless of the proximity to the source of contamination, and only considers entire spores in the 4–6 μm size range.

Conclusions and Implications

Understanding the factors that govern spore release, aerosolization, and transport allow prediction of potential exposure. *S. chartarum* spore emissions from gypsum board at low flow are directly proportional to airflow and inversely proportional to RH, which supports previous research with *Penicillium* and *Aspergillus*. The relationship between the culturable CFUs and total spores needs further investigation but suggests one reason that correlation between airborne field measurements (using culturable CFUs) and possible exposure is so difficult. Air filtration can be a helpful tool for minimising exposure and containing mold contamination while remediation is performed.

Acknowledgements

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References

1 Dearborn DG, Iwona Y, Sorenson WG, Miller MJ: Overview of investigations into pulmonary hemorrhage among infants in Cleveland, Ohio: Environ Hlth Perspect 1999;107(Suppl. 3): 495–499.

2 Vesper SJ, Vesper MJ: Stachylysin may be a cause of hemorrhaging in humans exposed to *Stachybotrys chartarum*: Infection and Immunity 2002;70(4):2065–2069.

3 Vesper SJ, Dearborn DG, Yike I, Allan T, Sobolewski J, Hinkley SF, Jarvis BB, Haugland RA: Evaluation of *Stachybotrys chartarum* in house of an infant with pulmonary hemorrhage: quantitative assessment before, during, and after remediation: J Urban Hlth 2000;77(1):68–85.

4 Sudakin DL: *Stachybotrys chartarum*: current knowledge of its role in disease: Medscape, www.medscape.com/viewarticle/408038, Feb 29, 2000, pE11.

5 Novotny W, Dixit A: Pulmonary hemorrhage in a infant following 2 weeks of fungal exposure: Arch Ped Adolescent Med 2000;154(3): 271–275.

6 Samet JM, Spengler JD: Indoor Air Pollution – A Health Perspective, John Hopkins University Press, Baltimore, MD, 1991.

7 Salvaggio J, Aukrust L: Allergy and clinical immunology. J Allergy Clin Immunol 1981;68(5).

8 Menetrez MY, Foarde KK, Ensor DS: An analytical method for the measurement of nonviable bioaerosols. J Air Waste Manage Assoc 2001;51:1436–1442.

9 Samson R, Hoekstra E: Introduction to Food Borne Fungi, Centraalbureau voor Schimmelcultures, P.O. Box 273, 3740 AG BAARN, The Netherlands, 1995.

10 Miller JD: Fungi as contaminants in indoor air. Atmos Environ 1992;26:2163–2172.

11 Sorenson WG, Frazer DG, Jarvis BB: Trichothecene mycotoxins in airborne conidia of *Stachybotrys atra*. Appl Env Microbiol 1987;53:1370–1375.

12 Foarde KK, Menetrez MY: Factors relating to the release of *Stachybotrys chartarum* spores from contaminated sources. Indoor Air, 2002;2:724–729.

13 VanOsdell D, Foarde K, Chang J: Design and Operation of a Dynamic Test Chamber for Measurement of Biocontaminant Pollutant Emission and Control. ASTM, 1996.

14 ASHRAE. ANSI/ASHRAE Standard 55-1992, Thermal Environmental Conditions for Human Occupancy, Atlanta: ASHRAE, 1992.

15 Foarde KK, VanOsdell DW, Menetrez MY, Chang JCS: Investigating the influence of relative humidity, air velocity, and amplification of the emission rates of fungal spores. Indoor Air '99 1999;2:507–512.

16 Sparks LE: IAQ Model for Windows, RISK Version 1.0, User Manual, EPA-600/R-96-037 (NTIS PB96-501929), Research Triangle Park, 1996.