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ABSTRACT

In an effort to better understand the relationship between different fungal sampling methods in the indoor environment, four methods were used to quantify mold contamination in 13 homes with visible mold. Swab, FSSST (Fungal Spore Source Strength Tester), and air samples were analyzed utilizing both the total spore count and colony forming unit (CFU) enumeration techniques. Dust samples were analyzed for culturable fungi only (CFU count), as no adequate method is available for the total spore enumeration. The relationships between the data obtained with the different sampling methods were examined using correlation analysis. Significant relationships were observed between the data obtained from swab and FSSST samples both by the total counting ($r=0.822$, $p<0.05$) and by the CFU counting ($r=0.935$, $p<0.01$). No relationships were observed between air and FSSST samples or air and dust samples. Percent culturability of spores for each sampling method was also calculated and found to vary greatly for all three methods (swab: 0.03 to 63%, FSSST: 0.1 to >100%, air: 0.7 to 79%). These findings suggest that reliance on one sampling or enumeration method for characterization of an indoor mold source might not provide an accurate estimate of fungal contamination of a microenvironment. Furthermore, FSSST sampling appears to be an effective measurement of a mold source in the field, providing a "worst case scenario" of potential mold spore release into the indoor air. However because of the small sample size of this pilot study, further research is needed to better understand the observed relationships in this study.

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Introduction

It has been estimated that 20 to 40% of homes in Northern Europe and Canada have mold contamination.[1] This number is likely to be much higher in tropical and subtropical countries.[2,3] In the United States, as many as 40% of homes have mold problems.[1,4] Various health effects, such as respiratory symptoms, allergic rhinitis, asthma, and hypersensitivity pneumonitis, are associated with mold exposure.[5-8] A case control study conducted in Europe suggested a relationship between asthmatic patients and increased mold and moisture problems in the home.[9] Other studies have shown that exposure to visible mold, or excessive moisture, which promotes mold growth lead to an increase in allergic symptoms.[6,8,10-16] Toxicity caused by exposure to the metabolites of certain molds have also been linked to health effects.[6,17] However, the relationship between specific health effects and the mold spore concentration has not been well-defined.[6] It has been criticized that the methodology for sampling and analysis is neither standardized, nor definitive.[18] Jarvis et. al.[18] have suggested that lack of a standard methodology is a primary cause for the poorly understood relationship between fungal exposures and health outcomes. Therefore, it is important to be able to identify and quantify the mold contamination levels in indoor environments.

One of two approaches is typically used to assess mold contamination with respect to fungal spore identification and enumeration: the colony forming unit (CFU) analysis, and the total spore count analysis. The CFU analysis, which is more common, deals with culturable fungal spores that are incubated on agar media. One advantage of CFU

analysis is the ability to identify colonies to the species level. Another advantage of this method is that a large reference database is available for proper identification of colonies.[19] However, there are several disadvantages of the CFU analysis. It is always selective, and the incubation period is long (over 7 days for some fungal species).[20-21] Also, CFU analysis can overlook fungal species that are not easily culturable, or might under-represent those fungal types that grow slowly because they are overtaken by faster growing colonies.[19,21-25] Kozak et. al.[26] demonstrated that although the level of culturable spores may be below the limit of detection, the total number of spores may be sufficient to cause respiratory symptoms. Some fungal species, such as the spores from *S. chartartum,* have been found to lose the ability to become cultured soon after they became airborne, however this does not appear to affect their allergenicity or toxicity.[3,26-27] In other words, some health effects, especially respiratory allergies, have been shown to be associated with the total spore count, rather than with the CFU count.[26,28] Similar to the culturable count, there are some advantages and disadvantages of the total spore count method. Two advantages are that (i) both viable and non-viable spores can be included, and (ii) the total count is less time consuming than the CFU analysis. Among disadvantages of this enumeration method, there are (i) masking effect, when the background matrix may mask small spores, (ii) high data variability when spore density is low, (iii) overestimation of large pigmented spores, and (iv) impossibility of performing the species-level identification.[19,29]

Other methods for fungal analysis include the use of surrogate markers that measure quantitative loads of fungal biomass, such as β-glucan and ergosterol. These indicator

methods are useful for providing general information about the amount of fungi in the environment, but are often not specific enough to relate to health outcomes because of their surrogate nature.[22] Recently, PCR (Polymerase Chain Reaction) and immunochemical methods have become available for fungal analysis.[27,29-34] There is currently, however, very little reference data available with these techniques.

Currently, there are numerous sampling methods available to measure fungal concentrations in the environment. Source sampling, which includes methods such as swab, tape, bulk, and dust, is commonly used to identify indoor fungi. These source sampling methods have been cited by the American Industrial Hygiene Association [35] as "necessary adjuncts" to air sampling, especially under conditions of low air movement, or when air sampling might result in false-negative findings. However, these surface-based methods cannot identify hidden sources of mold.[36] Swab and tape sampling are common methods of fungal exposure assessment through the source characterization, partially because of ease of collection. They are often used as tools for identification of fungi, but do not provide measures of exposure to airborne spores. Lastly, tape sampling is a quick method to investigate the presence of spores and mycelium on surfaces but cannot be used for quantitative analysis. Bulk samples include pieces of material such as wallboard, carpet, or return air filter, which are collected from the contaminated area to identify and find the relative concentration of mold in the sample.[36] Fungal spores can also be measured in settled dust sampled from the floor.[37-38] This method is usually utilized to evaluate long-term respiratory exposure to fungi, though the stability of microorganisms over time is questionable.[39-41] Flannigan et. al.[42] indicated that dust

may not adequately reflect human inhalation exposure, evidenced by his research findings that only a very small amount of re-aerosolized dust particles is of respirable size. Furthermore, Chew et. al.[39] found that culturable air and dust samples represent differing types of potential mold exposure, and thus, are not related indicators of exposure to mold. Settled dust can be analyzed by various techniques, such as CFU, β-glucan, and PCR. However a total spore enumeration is not available, in part because fungi in dust is masked by other particles.[33,39,43]

Air sampling is one of the most common methods used to assess fungal levels in indoor environments. Many studies have related human health effects, such as increases in allergic and asthmatic respiratory symptoms, to airborne fungal spores.[18,26,44-48] However, fungal spores have been found to exhibit varying patterns in their release into the air depending on several environmental factors.[18,39,44-48]

In an effort to link the mold source characterization and assessment of exposure to aerosolized fungal spores, several recent studies addressed the conditions necessary for fungal spore release from a mold source.[49-50] Two devices have been developed to measure the aerosolization potential of a visible fungal source, the Fungal Spore Source Strength Tester (FSSST) and the PFLEC (Particle Field and Laboratory Emission Cell).[51-54] Both of these devices utilize portable aerosolization chambers, in which spores are aerosolized from a fungal source and immediately collected into an air sampler.

The relationship between different fungal assessment methods has not been extensively characterized. Very little information is available on the comparison of the data obtained with the total and CFU enumeration of samples collected by a specific method, as well as the data collected by different sampling methods. Thus, a pilot-study was conducted to compare the data collected using four sampling methods in mold-contaminated homes. These methods include swab, FSSST, air, and dust sampling, and the first three were used to generate the total spore data and CFU data.

Methods

Twenty-six (26) homes with reported mold contamination were screened for this study in the greater Cincinnati metropolitan area. Thirteen (13) homes were selected for evaluation based on the size of the visible mold contamination (> 144 cm^2). Four types of sampling were performed on the selected homes: swab and dust (both representing the source), FSSST (represents the source aerosolization potential), and air sampling. Swab, FSSST and air methods were used as outlined by Sivasubramani et. al.[52]

Prior to the experiment, both relative humidity and indoor temperature were recorded using a traceable humidity/temperature pen (Fisher Scientific Company, Pittsburgh, PA). The surface moisture content of the test surface was measured with a Protimeter (GE Protimeter, Wilmington, DE) and expressed as a percentage of the mass of water in a given volume of a material [(wet mass – dry mass)×100/(dry mass)]. For a specific material, this percentage is calculated as a wood-equivalent value.

Swab sampling was performed on a 1 cm^2-area of the mold-contaminated surface (usually a wall). The area was thoroughly swabbed by using a sterile wet swab (Fisher Scientific Company, Pittsburgh, PA) to remove as much of the mold as possible and collected in a 0.5% Tween 80 solution (Sigma Chemicals Co., St. Louis, MO). The FSSST sampling unit is a closed, two-pump aerosolization chamber. A push-vacuum pump (11.5 L/min) produces an airflow that first passes through a HEPA filter (1244 HEPA capsule filter, PALL Gelman Laboratory, Ann Arbor, MI), then is directed

through a 112-hole orifice stage, passing over the mold-contaminated surface. The air is then drawn through a center orifice, into a BioSampler (SKC, Inc., Eighty Four, PA) at a rate of 12.5 L/min, using another vacuum pump. Each FSSST sample was collected for 10 min, which was shown to be sufficient to determine the spore aerosolization potential.[51-53] Simultaneously with the FSSST samples, the air samples were collected into the BioSampler (0.5% Tween 80 solution) using a vacuum-pull pump at a flow rate of 12.5 L/min for 10 min, which represents a short-term sampling. Dust sampling was performed using a canister-type vacuum cleaner (Filter Queen Majestic, Seven Hills, OH) fitted with a nozzle filter (pore size < 1 μm) (Filtration Group Industrial and Process, Joliet, Il). In every home, the dust sample was collected in the same room where the visible mold contamination was identified. Samples were vacuumed from a 2 m^2 area of carpet for 4 min. For hard floors, the dust sample was taken at a rate of 1 min/m^2.

The swab, air, and FSSST samples were analyzed for both culturable and total fungal spores. An aliquot of each sample was cultured on Malt Extract Agar (MEA), supplemented with streptomycin sulfate to inhibit bacterial growth.[55] Each sample was plated in triplicate, incubated for 7 days, and identified to the species level whenever possible. The dust samples were analyzed by the CFU method only, since there is no method currently available for the total fungal spore count of dust samples in part because spores are masked by other dust particles. For these samples, dust was suspended in a buffer solution containing 0.0425 g l^{-1} KH_2PO_4, 0.25 g $MgSO_4 x$ 7 H_2O l^{-1}, 0.008 g NaOH 0.02% (v/v) Tween 80. An aliquot of this solution (0.1 mL) was cultured on MEA treated with an antibiotic agent to inhibit bacterial growth. The

samples were incubated for 7 days and then identified to the species level whenever possible.

The procedures used for the total count of fungal spores in the swab, air, and FSSST samples have been fully described by Sivasubramani et. al.[52] Briefly, an aliquot of each sample was filtered onto a 13 mm mixed cellulose ester filter (Fisher Scientific Company, Pittsburgh, PA) (0.8 μm pore size) and then placed on a glass slide. Filters were dried overnight, then cleared by acetone vapor using a modified instant acetone-vaporizing unit (model Quickfix, Environmental Monitoring Systems, Charleston, SC). A 25 x 25 mm cover glass was mounted on the slide using glycerin jelly (Gelatin: 20 g, Phenol crystals: 2.4 g, Glycerol: 60 mL, Water: 70 mL). A light microscope (model Leitz Laborlux S, Leica Mikroskopie und Systeme GmbH, Germany, available through W. Nuhsbaum, Inc., McHenry, IL) at a magnification of 400X was used to identify and enumerate the collected fungal spores. For slides with a relatively high number of spores (> 50 spores per microscopic field), spores were enumerated in 20 microscopic fields; and for the slides with sparse deposit (< 50 spores per field), 40 microscopic fields were counted.

Correlational analyses were used to relate the number of spores collected by the different methods. Multiple comparisons were made between each collection type for both culturable and total spore counts. Scatterplots were generated using Sigma Plot (SPSS inc., Chicago, IL) and a correlation coefficient was calculated and tested for significance at $\alpha = 0.05$ for each relationship. The statistical significance of the correlation results was calculated using SPSS (SPSS inc., Chicago, IL). The percentage of culturable spores was determined for swab, FSSST, and air samples. Indoor air concentrations of fungi were

compared by utilizing the data on the outdoor levels measured on the same day around the Greater Cincinnati metropolitan area. These outdoor levels of fungi were measured using a Button Personal Inhalable Sampler, which collected particles on a mixed cellulose ester filter, at a flow rate of 4 L/min. This method has been fully described by Adhikari et. al.[56]

Results

Three types of surfaces with mold contamination were observed in the 13 homes in the study. Mold contamination on concrete surfaces occurred in 5 of the homes. Contamination of wood surfaces, including wood paneling and wood joist occurred in 4 homes. Contamination of drywall occurred in 4 homes, as well. Relative humidity ranged from 23 to 74% among the homes. Only 4 homes had relative humidity values over 50%. Surface moisture values ranged from 5.0 to 18.4% among the homes. The highest surface moisture (18.4%) occurred in the home contaminated with *Stachybotrys*. Temperatures in the homes ranged from 18.8 to 26.1°C.

Figure 1 presents boxplots for the concentration of spores with respect to the total and culturable fungal enumeration obtained from swab, FSSST, air (indoor and outdoor), and dust samples across all 13 homes. For 1 home, CFU analysis was not performed from any of the sampling methods, due to an oversight during the sampling period. In addition, swab samples from 2 homes and an air sample from 1 home were contaminated with bacteria, so CFU counts for fungal spores could not be determined. Lastly, culture samples taken from 1 home did not grow for neither the swab, nor air samples. In all of these homes, the total spore count was still obtained. Figure 1 shows that the median values of calculated spores over all the homes were an order of magnitude higher for both swab and FSSST in the total count method, as compared to the culturable count. The culturable count for these methods, however, had much higher variability. The median total spore level for indoor air was also higher for the total count method, but fell within the same order of magnitude as the culturable count. The variabilities of the indoor air

spore count levels were similar for both total and culturable methods. The median value measured in outdoor air (obtained only for the total count), was approximately an order of magnitude lower than the one determined in indoor air. The median culturable dust level was slightly higher than the air, and had similar variability.

Figures 2 and 3 show the number of homes in which each specific spore type was identified, with the total spore count (Figure 2) and CFU count (Figure 3). Not all spore types were found for each sampling method. *Aspergillus*, *Penicillium*, and *Cladosporium* were the most common fungal types identified in both the total and culturable counts.

Swab sampling from the visible mold sources (collected from contaminated walls) in 13 homes revealed 8 different types of fungal spores, as well as unidentified spores present in the total spore enumeration. For CFU analysis of swab samples taken from 9 homes, 7 spore types were identified. The occurrences of spore types identified in the total spore count were as follows: *Cladosporium* spores were found in 11 homes; *Aspergillus/Penicillium* spores were found in 6 homes; *Epiccocus* spores were found in 2 homes; *Alternaria*, *Ascospores, Chaetomium, Pithomyces*, and *Stachybotrys* spores were found in 1 home; and unidentified spores were found in 2 homes. The occurrences of culturable spore types were as follows: *Aspergillus* and *Cladosporium* spores were found in 6 homes; *Penicillium* spores were found in 5 homes; and *Epicoccus*, *Pithomyces*, *Stachybotrys*, and *Trichoderma* spores were each found in 1 home.

FSSST sampling from the visible mold sources in 13 homes revealed 7 different types of fungal spores present as unidentified spores in the total spore enumeration, as well. For

CFU analysis of FSSST samples taken from 12 homes, 8 spore types were identified. The occurrences of spore types identified by the total count were as follows: *Cladosporium* spores were found in 10 homes; A*spergillus/Penicillium* spores were found in 8 homes; and *Ascospores*, *Chaetomium, Epicoccus, Pithomyces*, and *Stachybotrys* spores were each found in 1 home. The occurrences of culturable spore types were as follows: *Aspergillus* spores were found in 11 homes; *Cladosporium* spores were found in 8 homes; *Penicillium* spores were found in 3 homes; *Paecilomyces* spores were each found in 2 homes; *Mucor*, *Epicoccum*, *Pithomyces*, and *Stachybotrys* were found in 1 home; and non-sporulating colonies were found in 4 homes.

Short-term air sampling conducted simultaneously with FSSST sampling in each of the 13 homes revealed 10 different types of fungal spores present for the total spore enumeration, as well as unidentified spores. For CFU analysis of the air samples taken from 10 homes, 9 spore types were identified. The occurrences of spore types with respect to their total count were as follows: *Aspergillus/Penicillium* spores were found in 12 homes; *Cladosporium* spores were found in 11 homes; *Basidiospores* spores were found in 5 homes; *Ascospores*, *Chaetomium*, and *Epicoccus* spores were found in 3 homes; *Periconia* was found in 2 homes; *Oidium/Erysiphe, Rust*, and *Stachybotrys* were each found in 1 home; and unidentified spores were found in 9 homes. The occurrences of fungal types with respect to CFU count were as follows: *Aspergillus* spores were found in 9 homes; *Penicillium* spores were found in 7 homes; *Cladosporium* spores were found in 7 homes; *Paecilomyces* spores were found in 3 homes; *Fusarium*, *Mucor,*

Pithomyces, and *Stachybotrys* colonies were found in 1 home; and non-sporulating colonies were found in 4 homes.

Dust sampling in 12 homes with visible mold contamination revealed 16 different fungal spore types (including non-sporulating colonies) through the CFU enumeration. The occurrences of culturable spore types were as follows: *Aspergillus* spores were found in 11 homes; *Cladosporium* spores were found in 10 homes; *Penicillium* spores were found in 9 homes; *Fusarium* spores were found in 7 homes; *Mucor* spores were found in 6 homes; *Alternaria* spores were found in 5 homes; *Paeciliomyces* and *Trichoderma* spores were found in 3 homes; *Gliocladium* and *Rhizopus* spores were found in 2 homes; *Epicoccus*, *Pithomyces*, *Sporthrix*, *Stachybotrys*, and *Syncephalastrum* were found in 1 home; and non-sporulating colonies were found in 5 homes.

Correlations between different collection methods were calculated for both total spore and CFU enumeration techniques (Figure 4). Total spore correlations were performed comparing the data obtained with the FSSST technique to both short-term air and swab measures. Since multiple types of fungi were collected at each home, comparisons were made wherever data points could be matched by fungal types. A significant correlation coefficient was observed between the FSSST and swab collection techniques. No significant relationship was observed between the short-term air and FSSST techniques for total spore enumeration.

Correlations were also determined wherever data points could be matched by fungal type with respect to the CFU enumeration (see Figure 4). Again, the only significant correlation was observed between the FSSST and swab collection techniques. There was no significant relationship observed between the short-term air and dust techniques, nor between air and FSSST techniques for CFU enumeration.

The percentage of culturable spores among the total counts obtained with swab, FSSST, and air sampled was calculated for each home. The results of this calculation are presented in Table 1. The culturable fungal fraction ranged from 0.03 to 63% in swab samples; FSSST samples revealed the culturability range of 0.1 to over 100% of culturable spores; and short-term air samples showed culturability of 0.7 to 79%. No information on the viability in dust samples was obtained because the total count was not available.

A comparison was made within homes to determine which type of sampling produced the highest percentage of culturable spores. In 6/11 (55%) homes, the air samples showed the highest spore culturability. The FSSST samples showed the highest culturability in 4 homes (36%). Swab samples revealed the highest culturablity in only 1 home (9%).

Discussion

The results in Figure 1 comparing the swab and FSSST methods showed that the median levels of spores released by the swab method were much greater than those released by the FSSST. These ranges are similar to those which we observed in our recent study of the FSSST performance with the median indoor total spore counts being about one order of magnitude higher than the outdoor levels.[52] Shelton et.al. [57] collected both indoor and outdoor mold samples using a culturable count method and found that the indoor levels were lower than those determined outdoors. Our indoor levels were most likely higher due to the presence of visible mold in housing. Though the indoor and outdoor air samples were collected with different methods (Biosampler and Button Sampler, respectively), both methods have been found to have high collection efficiency in the fungi particle range (<5μm).[56,58-59]

The swab samples showed greater variability of the CFU count compared to the total count. This large variation in enumeration type was seen in neither of the other two methods (FSSST or air). This is possibly due to the fact that only one swab sample was taken in an area of 1 cm^2. Perhaps the size of the swab sample led to greater variability of culturable spores determined by this method. In future studies, more swab samples should be taken when comparing swab to other methods.

Aspergillus, Penicillium, and *Cladosporium* spores were the most common types of fungi found in this study for both enumeration methods. These spore types are among the most predominant in the United States, and are generally considered to have indoor

origins.[57,60] In the total spore count method, *Cladosporium* spores were found in approximately the same number of homes by each sampling method. *Aspergillus/Penicillium* spores were identified in 6 of 13 homes for the swab method and 8 of 13 homes for the FSSST method. In both cases when *Aspergillus/Penicillium* spores were identified in the FSSST samples but not the swab, these spores represented a small fraction of the total spore count obtained by the former method. Perhaps these spores were also present in the swab samples, but were masked by the more prominent spore type. *Aspergillus/Penicillium* spores were also identified in 12 of the 13 homes during air sampling. Furthermore, all of the spore types identified by the total count were found in either equal amounts or more often in the air samples as compared to the swab and FSSST samples. Hyvarinen et. al.[61] reported similar results, namely, that more fungal species were identified using air sampling than by swab sampling. The investigators suggested that this could be due to the influence of unidentified indoor sources, or outdoor mold sources.

For the CFU analysis, the FSSST showed a greater number of homes containing both *Aspergillus* and *Cladosporium* culturable spores than did the swab or air sampling methods. *Aspergillus* colonies appeared in the FSSST samples in 5 more homes, when compared to the swab. It must be noted, however, that for 3 of these homes, the swab CFU samples were either contaminated, or did not grow. For the other 2 homes, *Aspergillus* spores represented a small fraction of the CFU count in the FSSST samples. It is possible that these spores were present in the swab samples, but did not grow due to the much higher concentration of other spore types, which might have overgrown the

Aspergillus spores. As previously mentioned, it is also possible that the small size of the swab sample was not fully representative of the source contamination when compared to the FSSST, which samples a much larger area. This explanation is also valid, when comparing the samples from 2 homes, among which the FSSST revealed *Cladosporium*, but the swab method did not. When comparing the culturable FSSST and air samples, it should be reminded that the FSSST is designed to assess a "worst-case scenario" for spore aerosolization.[51-52] Thus the FSSST induced culturable spore release in the homes where sporulation was not yet occurring by natural means, and thus was not detected in the air. Culturable spores in the dust were usually found in either equal or greater amounts compared to the other three methods. This supports the hypothesis that dust acts as a long-term sink for fungal spores. [39] Futhermore, 3 different mold types (*Fusarium*, *Mucor* and *Alternaria*) were found in 5 or more homes in the dust, but appeared one time or less in the other sampling methods. Perhaps, these fungal types represent the outdoor sources, suggesting the spore penetration and subsequent deposition on the floor.

Figure 4 shows the relationship between the different sampling methods for both total and CFU counts. A statistically significant relationship was observed between the FSSST and swab for both enumeration methods. This was an expected result, since both techniques measure the fungal source. There was no observed relationship between the FSSST and air level of fungi for either of the enumeration methods. Duchaine and Meriaux[62] reported that the number of mold sources in a home was significantly related to air CFU levels, suggesting an association between air and source samples in homes with visible mold contamination, and recommended that both sampling types are

necessary for a complete assessment of molds. Our study went a step further to quantitatively compare mold levels at the source, to those found in the air. We did not observe a relationship between these two methods. There are two possible explanations for this. First, spore release from fungal colonies is sporadic, and short- term air sampling might not accurately represent airborne levels.[39,52] Second, fungal spores sampled from the indoor air represent a mixture of spores from other potential indoor sources (other mold contamination, including non-identified growth on indoor surfaces and inside the ventilation system, as well as from the re-aerosolized dust). Outdoor sources may also contribute considerably, thus masking or otherwise under-representing spores released from the identified mold source. The points on these graphs were plotted whenever fungal types could be matched across sampling types. It would be expected that correlation values would be less, if all fungal types had been taken together.

Results on dust sampled from the carpet (or floor) were also compared with the results on samples in the room with mold contamination. This comparison was made because dust is often used as a measure of exposure to fungi, due to the potential re-aerosolization of dust particles into the air.[37-39] Again, no relationship was observed between these measures. Similar to the findings reported by Chew et. al.,[39] many more types of fungi were identified in the dust, as compared to the indoor air. Although dust has been recognized as a long-term reservoir for fungi, there appears to be little potential for re-aerosolization of fungi from indoor dust, as evidenced by this study and other investigations.[39,42,63] One potential factor that has not been considered thus far, is the inability to analyze dust by the total spore enumeration method. The dust samples

analyzed only by CFU method, leave the non-culturable fraction unknown. As stated previously, it has been suggested that allergic reaction to fungi is generally independent of culturability of spores.[26] It is then imperative, that a total spore enumeration method for fungal spores in dust be developed.

The data collected in this study allowed us to examine the relationship between total and CFU spore counts obtained by each of the three sampling methods (swab, FSSST, and air). Each method resulted in a wide range of culturability of spores both between and within homes. This finding is of particular interest, because a number of fungal sampling methods rely solely on the CFU enumeration technique. The results of this study support previous reports that this reliance might grossly underestimate the number of spores present in a sample, which might potentially lead to an underestimation of the severity of mold contamination.[19] The culturability of spores is dependent on a number of factors including spore type, temperature, and type of agar. Furthermore, since culturability is not generally linked to allergenic respiratory symptoms, these symptoms may still occur when the CFU enumeration technique generates results below the limit of detection.[26] In one FSSST sample, the percent culturable spores was found to be greater than 100% (125%). It has been hypothesized, though not tested, that this is due to the release of mycelial fragments, which could potentially grow to form new colonies, but would not be counted as spores.

Selecting appropriate methods for sampling fungal spores in indoor environments is crucial, in order to link the human exposure and disease caused by fungi. It has been

argued that the lack of standardized and definitive methods for mold sampling is a primary cause for the poorly understood relationship between mold exposure and health outcome.[18] Generally, air sampling has been a commonly used method to assess fungal exposure, and has also been described as the most representative of human respiratory exposure.[28,64-68] However, this study has demonstrated that short-term air sampling may not be an indicative measure of mold contamination in the indoor environment, as the number of spores released by the source (FSSST) did not relate to the airborne spore concentration. This was the case, even though the indoor mold contamination levels were approximately an order of magnitude higher than the outdoor levels, as seen in Figure 1. All of the environments chosen in this study had visible mold contamination, and multiple sampling methods were used for its quantification. It can be argued that in this type of environment, source testing would be the obvious choice for a sampling method. Furthermore, it could be suggested that if the mold source has been identified, there is no reason to sample, but instead to simply clean the contaminated area. At the same time, if the exposure to fungal spores is to be assessed in the presence of an identified mold source, short-term air sampling does not seem to be predictive of the source, even when the air sample is taken in the same room where the source was identified.

Conclusion

The results of this study suggest that reliance on one sampling or enumeration method for characterization of an indoor mold source might not provide an accurate estimate of fungal contamination of a microenvironment. As shown by other investigations, multiple sampling techniques are suggested when attempting to assess indoor mold contamination. The exclusive use of a CFU enumeration technique must be performed with the understanding that it might drastically underestimate the quantity of mold in the indoor environment. Additionally, culturable spores alone are not responsible for adverse health effects associated with mold exposure.

The relationships between the data obtained with the four different sampling methods were examined using correlation analysis. Significant relationships were observed between the data from swab and FSSST samples both by the total counting ($r=0.822$, $p<0.05$) and by the CFU counting ($r=0.935$, $p<0.01$). No relationships were observed between the data from air and FSSST samples or air and dust samples. Percent culturability of spores for each sampling method was also calculated and found to vary greatly for all three methods (swab: 0.03 to 63%, FSSST: 0.1 to >100%, air: 0.7 to 79%). FSSST sampling appears to be an effective way to assess the mold source in the field, providing a "worst case scenario" of potential mold spore release into the indoor air. However because of the small sample size of this pilot study, further research is needed to better understand the observed relationships in this study.

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TABLE I. Comparison of Culturability (culturable count/total count x 100%) Between Sampling Methods in 12 Homes. (N/A=sample was either contaminated or no microbial growth was observed).

Home	Swab	FSSST	Air
1	N/A	1.2	7.9
2	N/A	2.1	78.7
3	21.3	6.9	8.9
4	4.1	36.4	0.7
5	0.7	12.3	48.6
6	1.2	3.5	9.0
7	0.3	0.1	1.7
8	60.1	65.2	8.9
9	0.03	0.3	1.2
10	N/A	2.8	N/A
11	8.8	125	15
12	63	65	N/A

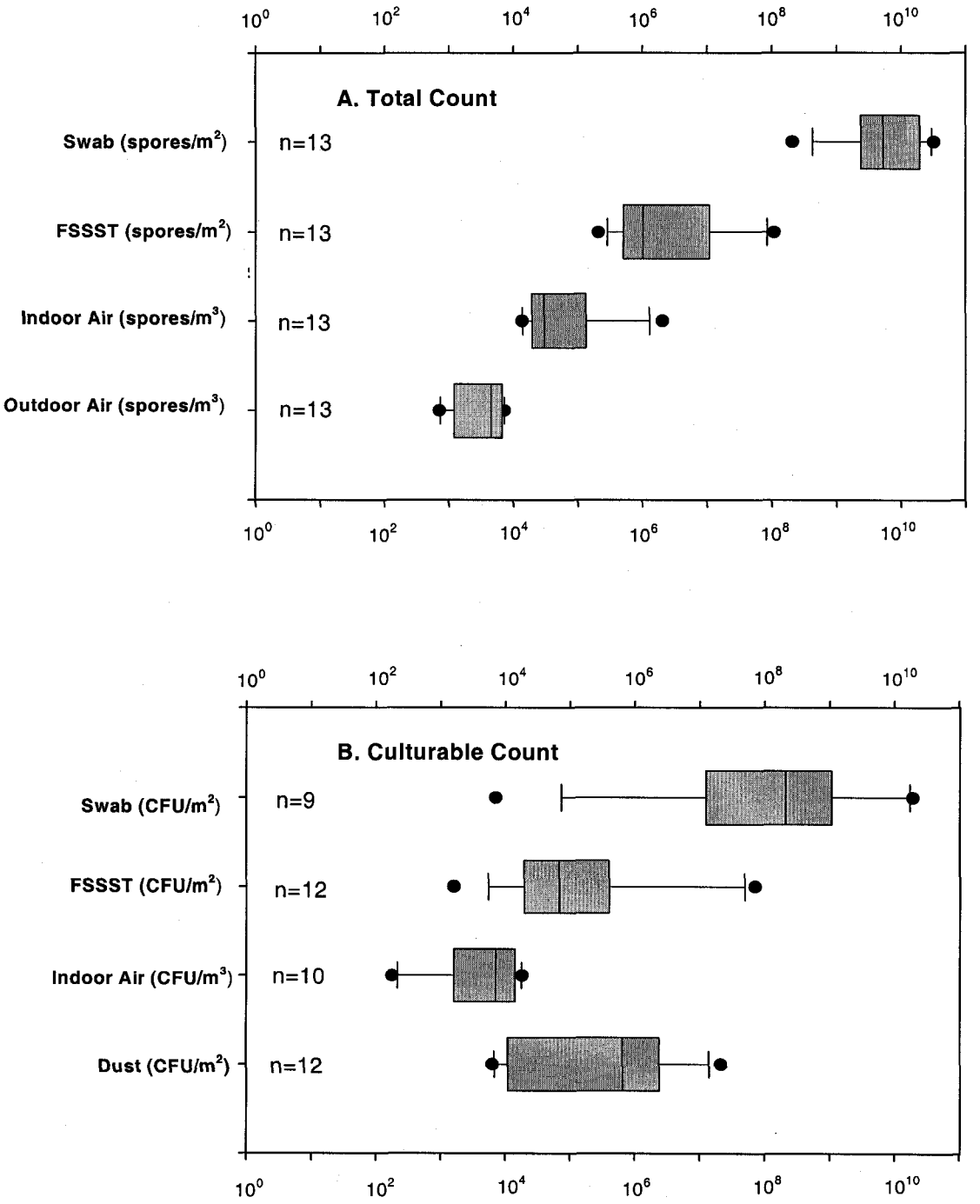

FIGURE I. Percentile and median values of concentrations of spores and colony forming units across 13 homes. The boxplot shows the following: horizontal lines from left, 5%, 25%, 50%, 75%, 95%, percentiles; symbol • shows the range of data; n: number of homes represented in each sampling method.

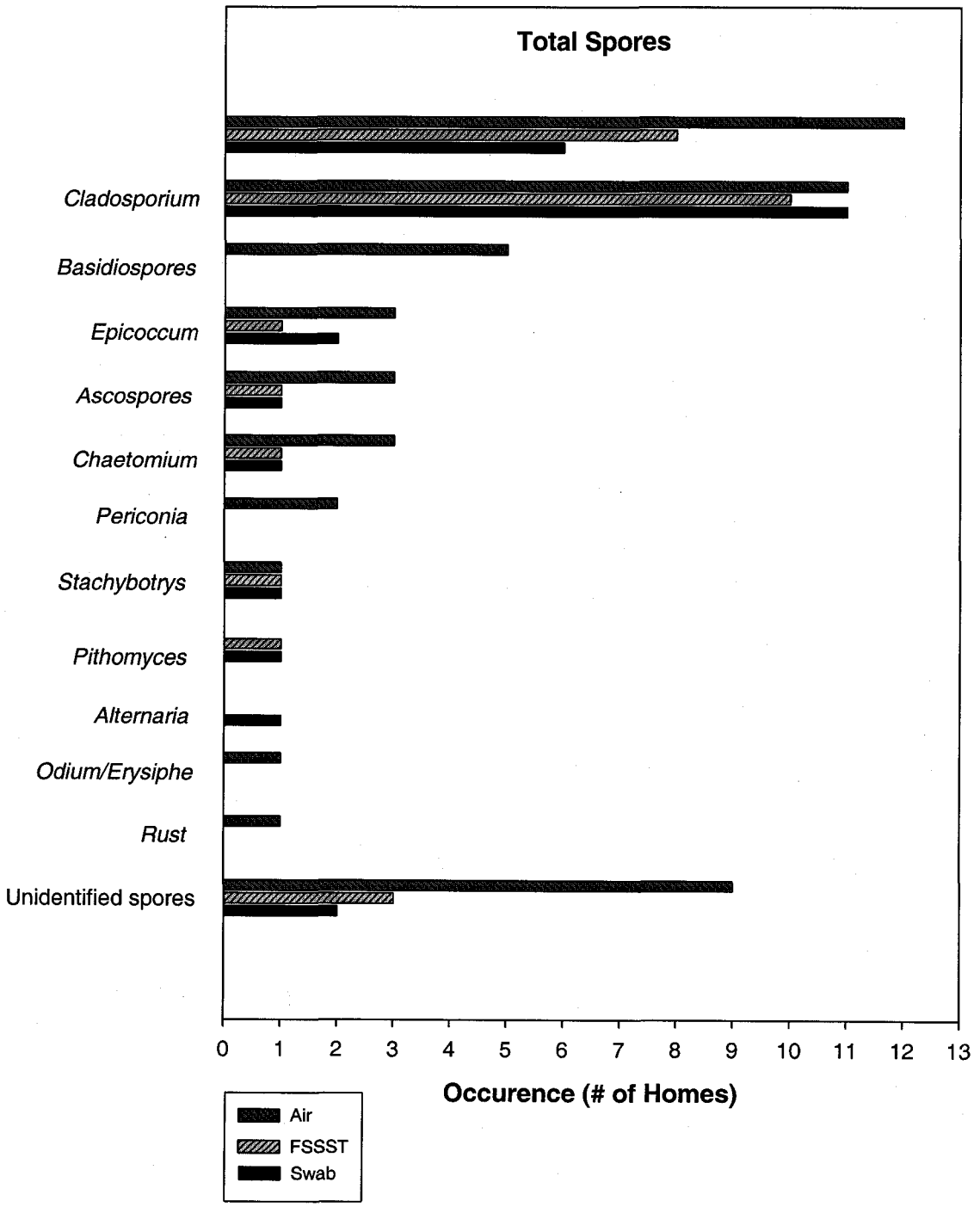

FIGURE II. Spore types identified by total spore enumeration in 13 homes.

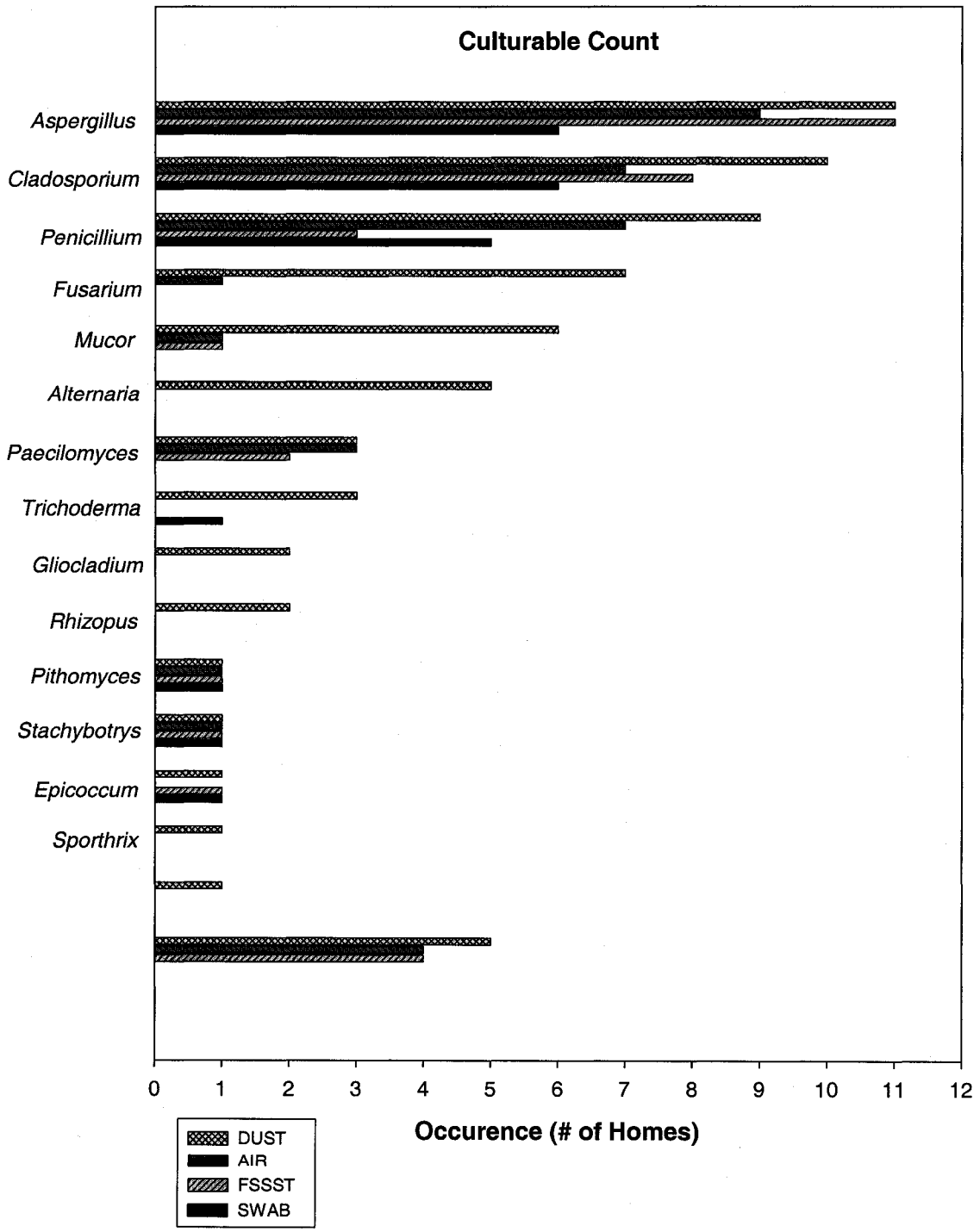

FIGURE III. Spore types identified by Culturable Count in 12 homes.

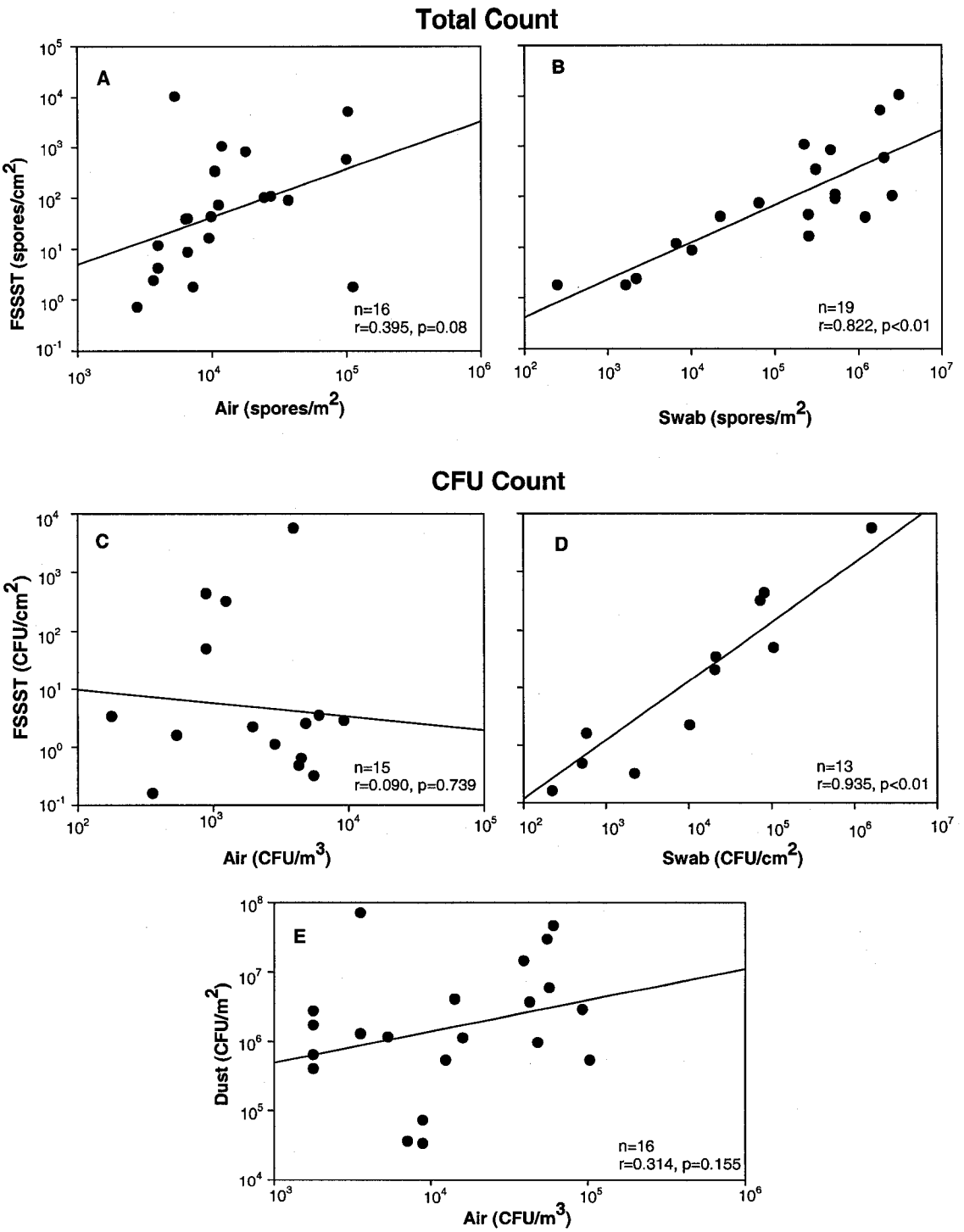

FIGURE IV. Correlations between the data obtained by different measurement methods for both total spore (A,B) and CFU enumerations (C,D,E).

Appendix

Sampling	type	Spore	Avg Conc./field	Total conc of filter	Conc in sample (20 ml)	Total Air (Spores/m3)	Total Fsst (spores/cm2)	Total Swab (spores/cm2)	Date
Air	total count	Cladosporium	3.8930	1923.121344	12821.45	102730			6/30/2003
FSSST	total count	Cladosporium	2.1080	1041.48	694668.36		4989		6/30/2003
Swab	total count	cladosporium	554.9700	274100.4494	1827428			1827428	6/30/2003
Air	total count	Aspergilli	3.7857	1869.761905	12465.1	99720.6349			7/15/2003
Air	total count	Periconia	0.0714	35.2785265	235.2	1881.52141			7/15/2003
FSST	total count	Aspergilli	24.0000	11853.58491	79023.9		567.5373411		7/15/2003
Swab	total count	Alternaria	0.0750	37.04245283	246.9			246.9496855	7/15/2003
Swab	total count	Aspergillus	619.8000	306118.8302	2040792.2			2040792.201	7/15/2003
Swab	total count	Cladasporium	7.2000	3556.075472	23707.2			23707.16981	7/15/2003
Swab	total count	Epicoccus	0.0250	12.34748428	82.3			82.31656184	7/15/2003
Air	total count	Aspergilli	0.4250	209.9072327	1399.4	11195.0524			7/25/2003
Air	total count	Basidiospores	0.0500	24.69496855	164.6	1317.06499			7/25/2003
Air	total count	Cheatom	0.0750	37.04245283	246.9	1975.59748			7/25/2003
Air	total count	Cladosporium	0.2500	123.4748428	823.2	6585.32495			7/25/2003
Air	total count	Unknown	0.0750	37.04245283	246.9	1975.59748			7/25/2003
FSST	total count	Aspergilli	3.1000	1531.08805	10207.3		73.30690656		7/25/2003
FSST	total count	Cladosporium	0.3667	181.0964361	1207.3		8.670709378		7/25/2003
Swab	total count	Aspergilli	19.6000	9680.427673	64536.2			64536.18449	7/25/2003
Swab	total count	Cladosporium	3.1000	1531.08805	10207.3			10207.25367	7/25/2003
Air	total count	Aspergillus	4.2500	2099.072327	13993.8	111950.524			8/1/2003
Air	total count	Cladosporium	1.4000	691.4591195	4609.7	36877.8197			8/1/2003
Air	total count	Epicoccus	0.4500	222.254717	1481.7	11853.5849			8/1/2003
Air	total count	Unidentified	0.2000	98.77987421	658.5	5268.25996			8/1/2003
FSST	total count	Aspergillus	0.0750	37.04245283	246.9		1.773554191		8/1/2003
FSST	total count	Chaeto	0.1000	49.38993711	329.3		2.364738921		8/1/2003
FSST	total count	Cladosporium	3.8000	1876.81761	12512.1		89.860079		8/1/2003
FSST	total count	Unknown	0.1500	74.08490566	493.9		3.547108382		8/1/2003
Swab	total count	Aspergillus	0.0750	37.04245283	246.9			246.9496855	8/1/2003
Swab	total count	cheatom	0.1250	61.73742138	411.6			411.5828092	8/1/2003
Swab	total count	Cladosporium	158.9000	78480.61006	523204.1			523204.0671	8/1/2003
Air	total count	Ascospores	0.2000	98.77987421	658.5	5268.25996			8/8/2003
Air	total count	Aspergillus	75.0400	37062.20881	247081.4	1976651.14			8/8/2003
Air	total count	Basidiospores	0.1333	65.85324948	439.0	3512.17331			8/8/2003
Air	total count	Cheato	0.3333	164.6331237	1097.6	8780.43326			8/8/2003
Air	total count	Cladosporium	1.0400	513.6553459	3424.4	27394.9518			8/8/2003
Air	total count	Unknown	0.1200	59.26792453	395.1	3160.95597			8/8/2003
FSST	total count	Cladosporium	4.6250	2284.284591	15228.6		109.3691751		8/8/2003
FSST	total count	Unknown	0.1750	86.43238994	576.2		4.138293112		8/8/2003
Swab	total count	Cladosporium	158.8400	78450.9761	523006.5			523006.5073	8/8/2003

Total Count

Sampling	type	Spore	Avg Conc./field	Total conc of filter	Conc in sample (20 ml)	Total Air (Spores/m3)	Total Fsst (spores/cm2)	Total Swab (spores/cm2)	Date
Air	total count	Aspergillius	0.3611	178.3525507	1189.0	9512.13604			9/24/2003
Air	total count	Basidiospores	0.0278	13.71942697	91.5	731.702772			9/24/2003
Air	total count	Cladosporium	0.1389	68.59713487	457.3	3658.51386			9/24/2003
Air	total count	Unknown	0.0556	27.43885395	182.9	1463.40554			9/24/2003
FSST	total count	Ascospores	0.0750	37.04245283	246.9		1.773554191		9/24/2003
FSST	total count	Aspergillius	0.7000	345.7295597	2304.9		16.55317245		9/24/2003
FSST	total count	Cladosporium	0.1000	49.38993711	329.3		2.364738921		9/24/2003
Swab	total count	Ascospores	1.5333	757.312369	5048.7			5048.749126	9/24/2003
Swab	total count	Aspergillius	76.8000	37931.4717	252876.5			252876.478	9/24/2003
Swab	total count	Cladosporium	0.6667	329.2662474	2195.1			2195.108316	9/24/2003
Air	total count	Aspergillus	0.1250	61.73742138	411.6	3292.66247			10/3/2003
Air	total count	Cheato	0.0250	12.34748428	82.3	658.532495			10/3/2003
Air	total count	Cladosporium	0.9250	456.8569182	3045.7	24365.7023			10/3/2003
Air	total count	Unknown	0.0500	24.69496855	164.6	1317.06499			10/3/2003
FSST	total count	Cladosporium	4.3250	2136.11478	14240.8		102.2749583		10/3/2003
Swab	total count	Cladosporium	773.0000	381784.2138	2545228.1			2545228.092	10/3/2003
FSST	total count	Aspergillus	34.4667	17023.06499	113487.1		815.0466815		10/29/2003
FSST	total count	Cladosporium	14.1333	6980.444444	46536.3		334.2164342		10/29/2003
Air	total count	Aspergillus	0.6750	333.3820755	2222.5	17780.3774			10/29/2003
Air	total count	Cladosporium	0.4000	197.5597484	1317.1	10536.5199			10/29/2003
Air	total count	Oidium/Erysiphe	0.0500	24.69496855	164.6	1317.06499			10/29/2003
Air	total count	Perconia	0.0250	12.34748428	82.3	658.532495			10/29/2003
Air	total count	Unknown	0.0750	37.04245283	246.9	1975.59748			10/29/2003
Swab	total count	Aspergillus	140.6000	69442.25157	462948.3			462948.3438	10/29/2003
Swab	total count	Cladosporium	93.2000	46031.42138	306876.1			306876.1426	10/29/2003
Air	total count	Aspergillus	1.3500	666.7641509	4445.1	35560.7547			11/14/2003
Air	total count	Cladosporium	1.3250	654.4166667	4362.8	34902.2222			11/14/2003
Air	total count	Stachybotrys	0.2000	98.77987421	658.5	5268.25996			11/14/2003
FSST	total count	pithomyces	7.6000	3753.63522	75072.7	600581.635	539.160474		11/14/2003
FSST	total count	stachybotrys	144.8000	71516.62893	1430332.6	11442660.6	10272.42587		11/14/2003
Swab	total count	Pithomyces	12.1000	5976.18239	119523.6			119523.6478	11/14/2003
Swab	total count	stachybotrys	311.9000	154047.2138	3080944.3			3080944.277	11/14/2003
air	total count	asper/pen	0.1500	74.08490566	493.9	3951.19497			3/23/2004
air	total count	rusts	0.0250	12.34748428	82.3	658.532495			3/23/2004
air	total count	unknown	0.3750	185.2122642	1234.7	9877.98742			3/23/2004
fssst	total count	asper/pen	0.1750	86.43238994	576.2		4.138293112		3/23/2004
fssst	total count	unknown	1.8500	913.7138365	6091.4		43.74767004		3/23/2004
swab	total count	unknown	76.0667	37569.27883	250461.9			250461.8588	3/23/2004

Sampling	type	Spore	Avg Conc./field	Total conc of filter	Conc in sample (20 ml)	Total Air (Spores/m3)	Total Fsst (spores/cm2)	Total Swab (spores/cm2)	Date
air	total count	aspergilli/pen	0.2750	135.822327	905.5	7243.85744			4/13/2004
air	total count	ascospores	0.0750	37.04245283	246.9	1975.59748			4/13/2004
air	total count	cladosporium	0.4500	222.254717	1481.7	11853.5849			4/13/2004
air	total count	epicoccus	0.0250	12.34748428	82.3	658.532495			4/13/2004
air	total count	unknown	0.2000	98.77987421	658.5	5268.25996			4/13/2004
FSSST	total count	cladosporium	44.2000	21830.3522	145535.7		1045.214603		4/13/2004
swab	total count	cladosporium	68.0000	33585.15723	223901.0			223901.0482	4/13/2004
air	total count	asper/pem	0.1750	86.43238994	345.7	2765.83648			4/27/2004
air	total count	basidospores	0.0500	24.69496855	98.8	790.238994			4/27/2004
air	total count	cladosporium	0.4000	197.5597484	790.2	6321.91195			4/27/2004
air	total count	unknown	0.2250	111.1273585	444.5	3556.07547			4/27/2004
fssst	total count	asper/pem	0.0500	24.69496855	98.8		0.709421676		4/27/2004
fssst	total count	cladosporium	2.7750	1370.570755	5482.3		39.37290304		4/27/2004
swab	total count	cladosporium	608.4000	300488.3774	1201953.5			1201953.509	4/27/2004
air	total count	asper/pen	0.2750	135.822327	905.5	7243.85744			4/28/2004
air	total count	ascospores	0.0500	24.69496855	164.6	1317.06499			4/28/2004
air	total count	basidiospores	0.0500	24.69496855	164.6	1317.06499			4/28/2004
air	total count	cladosporium	0.1500	74.08490566	493.9	3951.19497			4/28/2004
air	total count	epicoccum	0.2500	123.4748428	823.2	6585.32495			4/28/2004
air	total count	unknown	0.2750	135.822327	905.5	7243.85744			4/28/2004
FSSST	total count	asper/pen	0.0750	37.04245283	246.9		1.773554191		4/28/2004
FSSST	total count	cladosporium	0.5000	246.9496855	1646.3		11.82369461		4/28/2004
FSSST	total count	epicoccum	1.7000	839.6289308	5597.5		40.20056166		4/28/2004
swab	total count	asper/pen	0.5000	246.9496855	1646.3			1646.331237	4/28/2004
swab	total count	cladosporium	2.0000	987.7987421	6585.3			6585.324948	4/28/2004
swab	total count	epicoccum	6.7500	3333.820755	22225.5			22225.4717	4/28/2004
swab	total count	unknown	0.7500	370.4245283	2469.5			2469.496855	4/28/2004

Sampling	type	Spore	Conc in sample (20 ml)	Total Air (Spores/m3)	Total Fsst (spores/cm2)	Total Swab (spores/cm 2)	Date
Air	**culture**	aspergillius	22.2222	177.7777778			7/15/2003
Air	**culture**	non sporulating	222.2222	1777.777778			7/15/2003
Air	**culture**	Penicillium	755.5556	6044.444444			7/15/2003
Dust	**culture**	alternaria	666.6667				7/15/2003
Dust	**culture**	aspergillius	4666.6667				7/15/2003
Dust	**culture**	glioclad	1333.3333				7/15/2003
Dust	**culture**	non spor	666.6667				7/15/2003
Dust	**culture**	Penicillium	126666.6667				7/15/2003
FSST	**culture**	aspergillus	466.6667		3.351527339		7/15/2003
FSST	**culture**	penicillium	488.8889		3.511123879		7/15/2003
Air	**culture**	Aspergillus	555.5556	4444.444444			7/25/2003
Air	**culture**	Cladisporium	1288.8889	10311.11111			7/25/2003
Air	**culture**	Paecilomyces	355.5556	2844.444444			7/25/2003
Air	**culture**	Penicillium	66.6667	533.3333333			7/25/2003
Dust	**culture**	Cladisporium	4666.6667				7/25/2003
Dust	**culture**	fusarium	2666.6667				7/25/2003
Dust	**culture**	non spor	666.6667				7/25/2003
FSST	**culture**	aspergillus	88.8889		0.63838616		7/25/2003
FSST	**culture**	paecilomyces	155.5556		1.11717578		7/25/2003
Air	**culture**	Aspergillius	688.8889	5511.111111			8/1/2003
Air	**culture**	Cladosporium	244.4444	1955.555556			8/1/2003
Air	**culture**	Mucor	88.8889	711.1111111			8/1/2003
Air	**culture**	Nonsporolating Colonies	111.1111	888.8888889			8/1/2003
Air	**culture**	Penicillium	711.1111	5688.888889			8/1/2003
Dust	**culture**	Aspergillius	66666.6667				8/1/2003
Dust	**culture**	Fusarium	46666.6667				8/1/2003
Dust	**culture**	Glicladium	13333.3333				8/1/2003
Dust	**culture**	non spor	6666.6667				8/1/2003
Dust	**culture**	Penicillium	13333.3333				8/1/2003
FSST	**culture**	Aspergillius	44.4444		0.31919308		8/1/2003
FSST	**culture**	Cladosporium	311.1111		2.234351559		8/1/2003
FSST	**culture**	Nonsporilating Colonies	577.7778		4.149510039		8/1/2003
Swab	**culture**	Aspergillius	2222.2222			2222.22222	8/1/2003
Swab	**culture**	Cladosporium	1028888.8889			1028888.89	8/1/2003
Swab	**culture**	Penicillium	57777.7778			57777.7778	8/1/2003
Swab	**culture**	Trichoderma	24444.4444			24444.4444	8/1/2003

Culturable Count

Sampling	type	Spore	Conc in sample (20 ml)	Total Air (Spores/m3)	Total Fsst (spores/cm2)	Total Swab (spores/cm 2)	Date
Air	**culture**	Aspergillius	1155.5556	9244.444444			8/8/2003
Air	**culture**	penicillium	600.0000	4800			8/8/2003
Dust	**culture**	Alternaria	666.6667				8/8/2003
Dust	**culture**	Aspergillius	6000.0000				8/8/2003
Dust	**culture**	Cladosporium	1333.3333				8/8/2003
Dust	**culture**	Mucor	1333.3333				8/8/2003
Dust	**culture**	non spor	6000.0000				8/8/2003
Dust	**culture**	Penicillium	2000.0000				8/8/2003
Dust	**culture**	Trichoderma	4666.6667				8/8/2003
FSST	**culture**	Aspergillius	400.0000		2.872737719		8/8/2003
FSST	**culture**	cladosporium	4866.6667		34.95164225		8/8/2003
FSST	**culture**	mucor	22.2222		0.15959654		8/8/2003
FSST	**culture**	non sporulating	111.1111		0.7979827		8/8/2003
FSST	**culture**	penicillium	355.5556		2.553544639		8/8/2003
Swab	**culture**	cladosporium	21311.1111			21311.1111	8/8/2003
Air	**culture**	Aspergllius	177.7778	1422.222222			9/24/2003
Air	**culture**	Cladosporium	66.6667	533.3333333			9/24/2003
Air	**culture**	Non sporulating	155.5556	1244.444444			9/24/2003
Air	**culture**	Penicillium	533.3333	4266.666667			9/24/2003
Dust	**culture**	Alternaria	3333.3333				9/24/2003
Dust	**culture**	Aspergillius	14000.0000				9/24/2003
Dust	**culture**	Cladosporium	4000.0000				9/24/2003
Dust	**culture**	fusarium	666.6667				9/24/2003
Dust	**culture**	Mucor	666.6667				9/24/2003
Dust	**culture**	paeciliomyces	2000.0000				9/24/2003
Dust	**culture**	penicilliun	12666.6667				9/24/2003
FSST	**culture**	Aspergillus	66.6667		0.47878962		9/24/2003
FSST	**culture**	Cladosporium	222.2222		1.595965399		9/24/2003
FSST	**culture**	penicillium	66.6667		0.47878962		9/24/2003
Swab	**culture**	Aspergillius	555.5556			555.555556	9/24/2003
Swab	**culture**	cladosporium	577.7778			577.777778	9/24/2003
Swab	**culture**	penicillium	511.1111			511.111111	9/24/2003

Sampling	type	Spore	Conc in sample (20 ml)	Total Air (Spores/m3)	Total Fsst (spores/cm2)	Total Swab (spores/cm 2)	Date
Air	**culture**	Aspergillus	200.0000	1600			10/3/2003
Air	**culture**	Cladopsoruim	22.2222	177.7777778			10/3/2003
Air	**culture**	Fusari	22.2222	177.7777778			10/3/2003
Air	**culture**	Paeciliomyces	44.4444	355.5555556			10/3/2003
Air	**culture**	Penicillium	44.4444	355.5555556			10/3/2003
Dust	**culture**	Aspergillus	9333.3333				10/3/2003
Dust	**culture**	cladosporium	5333.3333				10/3/2003
Dust	**culture**	fusari	3333.3333				10/3/2003
Dust	**culture**	Mucor	666.6667				10/3/2003
Dust	**culture**	penicillium	10666.6667				10/3/2003
Dust	**culture**	pithomyces	666.6667				10/3/2003
Dust	**culture**	Sprothrise	666.6667				10/3/2003
FSST	**culture**	Aspergillus	66.6667		0.47878962		10/3/2003
FSST	**culture**	Cladosporium	377.7778		2.713141179		10/3/2003
FSST	**culture**	non spor	66.6667		0.47878962		10/3/2003
Swab	**culture**	aspergillus	200.0000			200	10/3/2003
Swab	**culture**	Cladosporium	29622.2222			29622.2222	10/3/2003
Air	**culture**	Aspergillus	44.4444	355.5555556			10/29/2003
Air	**culture**	Non spor	22.2222	177.7777778			10/29/2003
Dust	**culture**	Alternaria	20000.0000				10/29/2003
Dust	**culture**	Aspergillius	440000.0000				10/29/2003
Dust	**culture**	clad	220000.0000				10/29/2003
Dust	**culture**	Fusarium	120000.0000				10/29/2003
Dust	**culture**	Mucor	6666.6667				10/29/2003
Dust	**culture**	non spor	133333.3333				10/29/2003
Dust	**culture**	paecilomyces	20000.0000				10/29/2003
Dust	**culture**	penici	300000.0000				10/29/2003
Dust	**culture**	Rhizopus	26666.6667				10/29/2003
Dust	**culture**	Syncephalas	13333.3333				10/29/2003
Dust	**culture**	Trichoderma	26666.6667				10/29/2003
FSST	**culture**	Aspergillus	22.2222		0.15959654		10/29/2003
FSST	**culture**	Cladisporium	111.1111		0.7979827		10/29/2003
FSST	**culture**	Non sporulating	44.4444		0.31919308		10/29/2003
FSST	**culture**	Paecilomyces	22.2222		0.15959654		10/29/2003
Swab	**culture**	Aspergillius	222.2222			222.222222	10/29/2003
Swab	**culture**	penicillium	444.4444			444.444444	10/29/2003
Swab	**culture**	unknown	1555.5556			1555.55556	10/29/2003

Sampling	type	Spore	Conc in sample (20 ml)	Total Air (Spores/m3)	Total Fsst (spores/cm2)	Total Swab (spores/cm 2)	Date
Air	**culture**	asp	155.5556	1244.444444			11/14/2003
Air	**culture**	clad	111.1111	888.8888889			11/14/2003
Air	**culture**	penicillium	22.2222	177.7777778			11/14/2003
Air	**culture**	pithomyces	66.6667	533.3333333			11/14/2003
Air	**culture**	stachy	488.8889	3911.111111			11/14/2003
Dust	**culture**	aspergillus	10666.6667				11/14/2003
Dust	**culture**	cladosporium	666.6667				11/14/2003
Dust	**culture**	Fusarium	2000.0000				11/14/2003
Dust	**culture**	penicillium	8000.0000				11/14/2003
Dust	**culture**	Rhi	666.6667				11/14/2003
Dust	**culture**	Stachy	285333.3333				11/14/2003
Dust	**culture**	Trichoderma	1333.3333				11/14/2003
FSST	**culture**	asper	44444.4444		319.1930799		11/14/2003
FSST	**culture**	Clad	60000.0000		430.9106579		11/14/2003
FSST	**culture**	pithomyces	84444.4444		606.4668518		11/14/2003
FSST	**culture**	stachy	793333.3333		5697.596476		11/14/2003
Swab	**culture**	Asper	73333.3333			73333.3333	11/14/2003
Swab	**culture**	clad	82222.2222			82222.2222	11/14/2003
Swab	**culture**	pithomyces	142222.2222			142222.222	11/14/2003
Swab	**culture**	stachy	1626666.6667			1626666.67	11/14/2003
air	**culture**	Cladosporium	22.2200	177.76			3/23/2004
FSSST	**culture**	aspergillus	22.2200		0.15958058		3/23/2004
swab	**culture**	aspergillus	22.2200			22.22	3/23/2004
Swab	**culture**	penicillium	44.4400			44.44	3/23/2004
Dust	**culture**	aspergillus	21333.3333				3/23/2004
Dust	**culture**	cladosporium	12666.6667				3/23/2004
Dust	**culture**	penicillium	7333.3333				3/23/2004
Dust	**culture**	mucor	666.6667				3/23/2004

Sampling	type	Spore	Conc in sample (20 ml)	Total Air (Spores/m3)	Total Fsst (spores/cm2)	Total Swab (spores/cm 2)	Date
FSSST	**culture**	Aspergillus	4133.3333		29.68495643		4/13/2004
Dust	**culture**	non spor	533.3333				4/14/2004
Dust	**culture**	aspergillus	133.3333				4/14/2004
Dust	**culture**	cladosporium	66.6667				4/14/2004
air	**culture**	aspergillus	88.8800	711.04			4/27/2004
air	**culture**	cladosporium	111.1000	888.8			4/27/2004
air	**culture**	non spor	44.4400	355.52			4/27/2004
Fssst	**culture**	cladosporium	70000.0000		502.7291008		4/27/2004
swab	**culture**	cladosporium	106000.0000			106000	4/27/2004
dust	**culture**	cladosporium	1066.6667				4/27/2004
dust	**culture**	aspergillus	533.3333				4/27/2004
dust	**culture**	fusarium	133.3333				4/27/2004
dust	**culture**	mucor	66.6667				4/27/2004
FSSST		cladosporium	888.8000		6.383223212		4/28/2004
FSSST		epicoccum	2888.6000		20.74547544		4/28/2004
FSSST		aspergillus	1111.0000		7.979029015		4/28/2004
swab		epicoccum	206666.6667			206666.667	4/28/2004
dust		aspergillus	4866.6667				4/28/2004
dust		cladosporium	1466.6667				4/28/2004
dust		penicillum	1000.0000				4/28/2004
dust		alternaria	266.6667				4/28/2004
dust		paecilomyces	466.6667				4/28/2004
dust		epicoccum	8866.6667				4/28/2004

species	concentration in 20 ml (50mg dust)	total weight of dust sample	CFU/sample	area vacuumed (m2)	CFU/m2	date of sample
alternaria	666.7	3662	48826.67	2	24413.33333	7/15/2003
aspergillius	4666.7	3662	341786.7	2	170893.3333	7/15/2003
glioclad	1333.3	3662	97653.33	2	48826.66667	7/15/2003
non spor	666.7	3662	48826.67	2	24413.33333	7/15/2003
Penicillium	126666.7	3662	9277067	2	4638533.333	7/15/2003
cladisporiur	4666.7	1207	112653.3	2	56326.66667	7/25/2003
fusarium	2666.7	1207	64373.33	2	32186.66667	7/25/2003
non spor	666.7	1207	16093.33	2	8046.666667	7/25/2003
Aspergillius	66666.7	4423	5897333	2	2948666.667	8/1/2003
Fusarium	46666.7	4423	4128133	2	2064066.667	8/1/2003
Glicladium	13333.3	4423	1179467	2	589733.3333	8/1/2003
non spor	6666.7	4423	589733.3	2	294866.6667	8/1/2003
Penicillium	13333.3	4423	1179467	2	589733.3333	8/1/2003
Alternaria	666.7	5970	79600	2.5	31840	8/8/2003
Aspergillius	6000.0	5970	716400	2.5	286560	8/8/2003
ladosporiu	1333.3	5970	159200	2.5	63680	8/8/2003
Mucor	1333.3	5970	159200	2.5	63680	8/8/2003
non spor	6000.0	5970	716400	2.5	286560	8/8/2003
Penicillium	2000.0	5970	238800	2.5	95520	8/8/2003
richoderm	4666.7	5970	557200	2.5	222880	8/8/2003
Alternaria	3333.333	2871	191400	2	95700	9/24/2003
Aspergillius	14000	2871	803880	2	401940	9/24/2003
ladosporiu	4000	2871	229680	2	114840	9/24/2003
fusarium	666.6667	2871	38280	2	19140	9/24/2003
Mucor	666.6667	2871	38280	2	19140	9/24/2003
aeciliomyce	2000	2871	114840	2	57420	9/24/2003
penicilliun	12666.67	2871	727320	2	363660	9/24/2003
Aspergillus	9333.333	3001	560186.7	5	112037.3333	10/3/2003
ladosporiur	5333.333	3001	320106.7	5	64021.33333	10/3/2003
fusari	3333.333	3001	200066.7	5	40013.33333	10/3/2003
Mucor	666.6667	3001	40013.33	5	8002.666667	10/3/2003
penicillium	10666.67	3001	640213.3	5	128042.6667	10/3/2003
pithomyces	666.6667	3001	40013.33	5	8002.666667	10/3/2003
Sprothrise	666.6667	3001	40013.33	5	8002.666667	10/3/2003

species	concentration in 20 ml (50mg dust)	total weight of dust sample	CFU/sample	area vacuumed (m2)	CFU/m2	date of sample
Alternaria	20000	6430	2572000	8	321500	10/29/2003
Aspergillius	440000	6430	56584000	8	7073000	10/29/2003
clad	220000	6430	28292000	8	3536500	10/29/2003
Fusarium	120000	6430	15432000	8	1929000	10/29/2003
Mucor	6666.667	6430	857333.3	8	107166.6667	10/29/2003
non spor	133333.3	6430	17146667	8	2143333.333	10/29/2003
aecilomyce	20000	6430	2572000	8	321500	10/29/2003
penici	300000	6430	38580000	8	4822500	10/29/2003
Rhizopus	26666.67	6430	3429333	8	428666.6667	10/29/2003
Syncephala	13333.33	6430	1714667	8	214333.3333	10/29/2003
Trichoderm	26666.67	6430	3429333	8	428666.6667	10/29/2003
aspergillus	10666.67	2334	497920	9.29	53597.41658	11/14/2003
ladosporiur	666.7	2334	31120	9.29	3349.838536	11/14/2003
Fusarium	2000.0	2334	93360	9.29	10049.51561	11/14/2003
penicillium	8000	2334	373440	9.29	40198.06243	11/14/2003
Rhi	666.7	2334	31120	9.29	3349.838536	11/14/2003
Stachy	285333.3	2334	13319360	9.29	1433730.893	11/14/2003
Trichoderm	1333.3	2334	62240	9.29	6699.677072	11/14/2003
aspergillus	21333.33	5379	2295040	5	459008	3/23/2004
cladsporiu	12666.67	5379	1362680	5	272536	3/23/2004
penicillium	7333.333	5379	788920	5	157784	3/23/2004
mucor	666.6667	5379	71720	5	14344	3/23/2004
non spor	533.3333	866	9237.333	2	4618.666667	4/14/2004
aspergillus	133.3333	866	2309.333	2	1154.666667	4/14/2004
cladsporiu	66.66667	866	1154.667	2	577.3333333	4/14/2004
cladsporiu	1066.667	1349	28778.67	4	7194.666667	4/27/2004
aspergillus	533.3333	1349	14389.33	4	3597.333333	4/27/2004
fusarium	133.3333	1349	3597.333	4	899.3333333	4/27/2004
mucor	66.66667	1349	1798.667	4	449.6666667	4/27/2004
aspergillus	4866.667	518	50418.67	2	25209.33333	4/28/2004
cladsporiu	1466.667	518	15194.67	2	7597.333333	4/28/2004
penicillum	1000	518	10360	2	5180	4/28/2004
alternaria	266.6667	518	2762.667	2	1381.333333	4/28/2004
paecilomyc	466.6667	518	4834.667	2	2417.333333	4/28/2004
epicoccum	8866.667	518	91858.67	2	45929.33333	4/28/2004

Environmental Characteristics of the Tested Homes

Date	Type of surface contamination	Relative Humidity (%)	Temperature (C)	Surface Moisture (%)
6/30/2003	Wood Joist	40	21.7	9
7/15/2003	Wood paneling	74.0	21.8	15.4
7/25/2003	Wood Paneling	55.0	22.3	10.2
8/1/2003	Concrete	68	24.9	21
8/8/2003	Plaster	41	26.1	5
09/24/03	Wood Paneling	54.0	23.2	9.7
10/03/03	Concrete	32.0	20.0	15.0
10/29/2003	concrete	32	20	11.3
11/14/2003	drywall	25	21.3	18.4
3/23/2004	concrete	23	18.8	13
4/13/2004	drywall	40	22.2	10
4/27/2004	drywall	38	23.2	14
4/28/2004	concrete	44	22.9	11

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Assessment of the aerosolization potential for fungal spores in moldy homes

Abstract The airborne fungal concentration measured with air samplers during specific time intervals may not adequately represent the indoor air quality because of the sporadic nature of spore release from sources. The conventional source evaluation (e.g. swab and tape sampling) characterizes the mold source but does not relate to the fraction of spores that can be aerosolized from a contaminated material. As an alternative to these methods, we have recently developed and laboratory-tested a novel Fungal Spore Source Strength Tester (FSSST). It allows assessing the potential of aerosolization of fungal spores from contaminated surfaces under the most favorable release conditions. In this study, the FSSST was used to characterize the release of spores from four building materials in mold-problem homes. The spores of different species were efficiently aerosolized by the FSSST, exhibiting a total spore release rate ranging approximately from 10^2 to 10^3 cm^2/min. For all tested materials, <2% of the spores on the contaminated surface were released during the tests. The airborne spore concentration estimated from the release rate data was found in most cases to be significantly greater than the concentration actually measured in these environments with simultaneous air sampling. The results suggest that the FSSST can be used for the assessment of maximum potential exposure to airborne spores released from identified sources in homes.

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Key words: Fungi; Moldy buildings; Spore release; Aerosol sampling; Source strength.

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Practical Implications

A recently developed FSSST was found to be suitable to measure the aerosolization potential of indoor fungal sources at the most favorable release conditions. The FSSST generates the data that allows assessing the strength of mold sources in homes with respect to their maximum ability to contaminate indoor air with fungi. The novel approach bridges two conventional methods, the air sampling and the direct source evaluation (e.g. swab sampling), thus providing a better representation of the airborne fungal exposure than these methods individually. The device prototype can be used for evaluating the effectiveness of environmental interventions by taking samples before and after the intervention. As a broader application, the FSSST can be utilized for assessing the release of various hazardous biological and non-biological particles from contaminated surfaces.

Introduction

Numerous health effects in homes, schools, and offices have been attributed to the fungal growth resulting from water damage or improper humidity in buildings (Dales et al., 1991a; Lacey and Crook, 1998; Wickman et al., 1992). The exposure to mold and dampness has been associated with respiratory symptoms (Dales et al., 1991b) and may particularly increase the risk of adverse respiratory health effects (Peat et al., 1998; Verhoeff and Burge, 1997).

The fraction of buildings with mold-contamination in the United States and Canada is about 36%, according to Spengler et al. (1993). Brunekreef et al. (1989) have reported that 20–40% of homes in Northern Europe and North America have mold problems. In the Netherlands and Finland, the mold and dampness in buildings were reported as 15 and 24%, respectively (Pirhonen et al., 1996; Verhoeff et al., 1990). The age of a building is an important factor affecting the fungal spore concentration in indoor air (Pasanen et al., 1992; Rand, 1999). Modern buildings are constructed with various types of materials that provide ecological niches with varying nutritional and temperature conditions. A variety of interactions between microorganisms may occur under these conditions. While practically all building materials can serve as a substrate for fungal growth when excess moisture is present, the growth and aerosolization rates may differ for different materials. The fungal growth mainly depends on the nutrient availability, alkalinity, porosity, and the water activity of the material. Water

activity is defined as the amount of free water in the material available for microbial growth and depends on the moisture absorbing potential of the growth material (Flanningan and Morey, 1996).

Wood and wood composites as well as materials with high starch content are capable of supporting mold growth at the lowest values of water activity (Hukka and Viitanen, 1999; Nielsen et al., 2000; Viitanen and Bjurman, 1995). Plasterboard reinforced with cardboard and paper fibers, as well as inorganic materials coated with paint or treated with additives that offer an easily degradable carbon source, are excellent substrate for molds. However, they support fungal growth only at relatively high water activity (Chang et al., 1995, 1996). Other inorganic materials with traces of organic ones seem to be able to support growth at very high water activity (Nielsen, 2003).

Variations in indoor humidity and temperature exert a profound influence on mold growth (Viitanen and Bjurman, 1995). The local differences in ventilation and surface temperature can generate microclimates with very high water activity. For this reason, a measurement of indoor relative humidity may not be sufficient to predict mold problems (Becker, 1984; Grant et al., 1989; Gravesen et al., 1999; Hukka and Viitanen, 1999). Water activity is the most important factor in determining whether or not mold growth is initiated on building materials (Ayerst, 1966; Galloway, 1935; Hukka and Viitanen, 1999; Rowan et al., 1999; Scott, 1957).

The release of fungal spores from their source is driven by the energy from external sources and may be significantly affected by environmental factors. The aerosolization caused by air currents seems to be the most prevalent mechanism for indoor fungi (Gregory, 1973; Madelin, 1994). For example, for *Aspergillus* and *Penicillium*, the air currents have been indicated as the principle physical factor causing the spore detachment and dispersion in indoor environments (Burnett, 1976). Zoberi (1961) found that the release of spores of different fungal species from agar surface is mainly a function of air velocity so that the velocity increase causes increase in the spore release rate. Pasanen et al. (1991) reported that the air velocity required for the spore release was dependent on the fungal type. Kildesø et al. (2003) demonstrated that the release of spores from the wet wallpapered gypsum board differs for different fungi under identical conditions. We found that the fungal spore release was affected by the air velocity above the surface, texture of the surface, and vibration of the contaminated material (Górny et al., 2001).

Air sampling and testing of building materials are often performed to identify the agents that are potentially responsible for health problems and to determine the level of exposure to these agents (Beguin and Nolard, 1994; DeKoster and Thorne, 1995; Icenhour and Levetin, 1997; Macher and Huang, 1991). In principle, the air sampling should be the most representative of human respiratory exposure (Burge, 1995). However, this method is often not representative for assessing a long-term exposure to airborne fungal spores because the sampling period is limited and the spore aerosolization is sporadic. In case the air samples are analyzed through the culture-based enumeration, low viability/culturability may be a reason that some species are not detected. The above factors as well as the lack of knowledge about the actual mechanisms of diseases are essentially responsible for a poor association between the health effects and the exposure assessment data generated from the analysis of air samples. As an alternative to the air sampling, some exposure studies are carried out through the direct evaluation of fungal sources (Flappan et al., 1999; Meyer et al., 2004). The currently available techniques such as bulk sampling, surface sampling and dust sampling allow the investigators to collect extensive information about the source, but cannot measure its aerosolization potential and, therefore, may not adequately represent the aerosol exposure. For instance, Chew et al. (2003) reported the air and dust represent different types of potential fungal exposures.

To bridge two conventional methods (the air sampling and the direct source evaluation), the Fungal Spore Source Strength Tester (FSSST) was recently developed (Grinshpun et al., 2002). The new method, in which the spores are aerosolized from the source into a small chamber and immediately collected from it with a bioaerosol sampler, was recently evaluated under controlled laboratory conditions (Sivasubramani et al., 2003). The FSSST prototype was found suitable for assessing the aerosolization potential of the fungal spores from the growth source. This non-destructive assessment of fungal contamination on building materials seems to be an important additional advantage of the FSSST. The objective of this study was to use the FSSST prototype for determining the rate and efficiency of fungal spore release from different contaminated building materials in mold-problem homes.

Materials and methods

Four residential homes with visibly mold-contaminated surfaces, including concrete, painted dry wall, particleboard and wooden joist, were selected in the Greater Cincinnati metropolitan area. Because of its climate conditions and frequent flooding, the area has a considerable number of water-damaged homes and a very high occurrence of respiratory allergies and asthma, especially in children. One mold-damaged room was tested in each home. The main criterion was that the room had at least 0.5 m^2 of the visible moldy surface with a uniform contamination to allow for testing with the swab method and by the FSSST. The

uniformity of the mold growth was checked with a hand-held magnifier lens. The test rooms were of about the same size (the room volumes ranged from 107 to 121 m^3).

Prior to the experiment, the indoor temperature and relative humidity were measured with a traceable humidity/temperature pen (Fisher Scientific Company, Pittsburgh, PA, USA). The surface moisture content of the test surface was measured with a Protimeter (GE Protimeter, Wilmington, MA, USA) and expressed as a percentage of the mass of water in a given volume of a material, (wet mass − dry mass) × 100/(dry mass). For a specific material, this percentage is calculated as a wood-equivalent value. Each building material, tested in this study, was characterized with respect to the initial spore contamination using the swab sampling method. A sample of approximately 1 cm^2 was taken with a sterile wet swab from four different places, randomly chosen on the test surface. Each sample was suspended in 20 ml of de-ionized, sterilized water with 0.05% Tween 80 (Sigma chemicals Co., St Louis, MO, USA) in a test tube. The swab sample suspensions were vortexed. Three milliliter of suspension was vacuum filtered onto a 13-mm mixed cellulose ester filter (0.8 μm pore size). The spore-containing filters were dried by placing them in sterile Petri plates at room temperature for 2 h. Dry filters were placed on a glass slide and cleared by acetone vapor utilizing a modified instant acetone-vaporizing unit (model Quickfix, Environmental Monitoring Systems, Charleston, SC, USA). A 25 × 25 mm cover glass was mounted on the slide using glycerin jelly (Gelatin: 20 g, Phenol crystals: 2.4 g, Glycerol: 60 ml, Water: 70 ml). The spores on the slide were counted by using a light microscope (model Leitz Laborlux S, Leica Mikroskopie und Systeme GmbH, Germany, available through W. Nuhsbaum, Inc., IL, USA) at a 400× magnification. For slides with a dense spore deposit (> 50 spores per microscopic field), the spores were enumerated in 20 microscopic fields; and the slides with sparse deposit (< 50 spores per field), 40 microscopic fields counted. The total spore count and the count of individual spore types were performed on each sample. The initial spore surface density, N_S, was determined based on the number of spores counted on a 1-cm^2 area from which the swab sample was taken (N_S was calculated for both the total and spore-type-specific counts).

Following the above-described direct source evaluation, the FSSST was applied to the test surface. The device is schematically shown in Figure 1. It consists of a cup like aerosolization chamber, which is applied to and held against the mold-contaminated surface. A push vacuum pump produces the airflow Q_{IN}^{FSSST} that passes through the HEPA filter (1244 HEPA capsule filter, PALL Gelman Laboratory, Ann Arbor, MI, USA). The flow Q_{IN}^{FSSST} is directed through 112-orifice stage at the bottom of the device

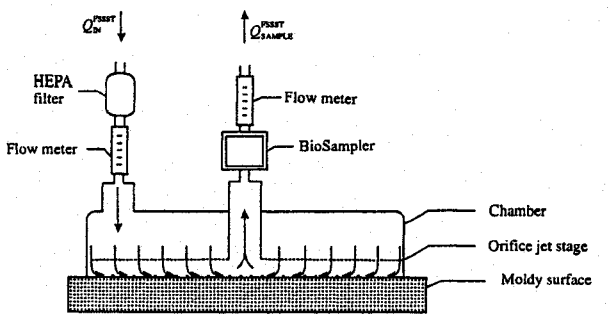

Fig. 1 Fungal spore source strength tester

creating air jets towards the contaminated surface. The air jet velocity that is approximately 13.6 m/s at the orifice decreases considerably towards the contaminated surface. The fungal spores, aerosolized by these air jets, are collected into the BioSampler (SKC Inc., Eighty Four, PA, USA). The sampler is located at the outlet and operated by a pull vacuum pump at a flow rate of Q_{IN}^{FSSST} = 12.5 l/min. The collection vessel is filled with a 20 ml suspension of de-ionized, sterilized water mixed with 0.05% Tween 80. The flow rate balance is set so that the Q_{IN}^{FSSST} is slightly lower than the Q_{IN}^{FSSST} (Δ = 1.0 l/min). As a result, no fungal spore contamination occurs in the ambient air during the FSSST operation. Some spores may penetrate into the aerosol chamber from the outside air environment but their number is rather low compared with the number of spores released from the source because of the difference in (i) flow rates ($\Delta \ll Q_{IN}^{FSSST}$) and (ii) micro environmental fungal concentration (air < mold source). The airflows at the FSSST's inlet and outlet are controlled with flow meters (model 2A17, Key Instruments, Trevose, PA, USA) calibrated with a Buck calibrator (A.P. Buck, Orlando, FL, USA).

The FSSST was applied to the mold-contaminated surface during four time intervals: t_{FSSST} = 5, 10, 15, and 20 min. Thus, four samples were collected on each surface. The device was thoroughly cleaned between the tests with 70% ethyl alcohol, and a separate sterile BioSampler was used for each test. Simultaneously with the use of the source tester, four indoor air samples were collected by the BioSampler (Q_{AIR}^{SAMPLE} 12.5 l/min) at about 2 m away from the source using the same sampling time intervals as for the FSSST. After the sampling, the collection fluid of each BioSampler (attached to the FSSST and acting as a stationary air sampler) was filtered through a mixed cellulose ester filter, which was subsequently cleared by acetone vapor and analyzed under the microscope as described above.

The spore release rate (R, per square centimeter and per minute), defined as the number of airborne spores aerosolized from 1 cm^2 of the test surface during one minute of the FSSST application, was determined as:

$$R = \frac{N_{FSSST}}{A \times t_{FSSST}} \quad (1)$$

where N_{FSSST} is the number of spores obtained in the BioSampler attached to the source tester, and A is the surface area subjected to the source testing ($A \approx 140$ cm^2). The airborne spore concentration was determined as

$$C_{AIR}^{MEASURED} = \frac{N_{AIR}}{Q_{SAMPLE}^{AIR} \times t} \quad (2)$$

where N_{AIR} is the number of spores obtained in the BioSampler operating as a stationary air sampler and t is the air sampling time. The measured spore release rate, R, can be used to assess the 'worst-case' scenario by estimating the airborne spore concentration as

$$C_{AIR}^{ESTIMATED} = \frac{R \times A_{CONTAMINATION} \times t_{FSSST}}{V_{ROOM}} \quad (3)$$

where CONTAMINATION is the area of the contaminated surface and V_{ROOM} is the volume of the room. This assumes that (i) t_{FSSST} is sufficiently long to expect that most of the spores, which are ready to release, will become airborne, and (ii) no reduction of the aerosol concentration because of gravitational settling and other mechanisms is considered in this 'worst-case' scenario.

The relative efficiency of spore release [E_R (%)] was defined as the number of spores released from 1 cm^2 during the time of the FSSST's application to the initial number of spores on the area of 1 cm^2 determined by the swab sampling method. The E_R values were calculated as:

$$E_R = \frac{R \times t_{FSSST}}{N_S} \times 100\% \quad (4)$$

Results

The highest total initial spore surface density, $N_S = (19.2 \pm 3.7) \times 10^5$/cm^2, occurred on the wooden joist, followed by particleboard [$(14.6 \pm 5.9) \times 10^5$/cm^2], painted drywall [$(6.9 \pm 2.7) \times 10^5$/cm^2], and concrete [$(3.6 \pm 0.6) \times 10^5$/cm^2]. In a wood-based material, readily available nutritional condition and the capillary action of water absorption (Pasanen et al., 2000) are likely to facilitate considerable fungal growth.

The identification analysis of swab samples, taken from the four tested building materials, revealed six spore types. The *Aspergillus/Penicillium* group was detected on all tested materials, except the wooden joist. *Chaetomium* spores were detected on painted drywall and particleboard. Spores of *Cladosporium* were found on concrete, painted drywall and wooden joist. Basidiospores and *Stachybotrys* were found only on painted drywall. The greatest number of fungal types was identified in samples taken from the painted drywall. The following air temperature and relative humidity levels were recorded: approximately 21°C and 40% in homes where the particleboard and wooden joist were tested, 23°C and 20% in the home with mold-contaminated painted drywall, and 17°C and 69% in the home with moldy concrete surface. The surface moisture content observed in the tested materials was within a relatively narrow range of 9–15%.

The airborne concentrations of fungal spores observed in the tested homes ranged approximately from 10^4 to 10^5/m^3. It was higher in the homes where mold-contaminated concrete and painted dry wall were tested [$C_{AIR}^{MEASURED} = (1.7 \pm 4.3) \times 10^4$/m^3 and $(10.8 \pm 0.6) \times 10^4$/m^3, respectively] and lower in the homes with particleboard and wooden joist [$C_{AIR}^{MEASURED} = (1.7 \pm 0.6) \times 10^4$/m^3 and $(1.4 \pm 0.2) \times 10^4$/m^3, respectively]. These airborne spore concentrations are of the same order of magnitude as those reported by Rautiala et al. (1996) from their aerosol measurements performed in moldy buildings.

All the fungal spore types identified from the swab samples were also found in the air samples. In addition, the air sample analysis revealed some fungal types that were not found in the swab samples, suggesting the influence of outdoor sources or/and non-identified indoor sources. Hyvärinen et al. (1993) also observed some fungal genera in air samples while these genera were not found in the surface samples.

Figure 2 shows the spore release rates obtained with the FSSST for each building material, when the source tester was applied during 5, 10, 15, and 20 min. It is seen from Figure 2 that the spores were efficiently aerosolized by the FSSST from all four tested materials with a total spore release rate ranging approximately from 10^2 to 10^3/cm^2/min. While the average R-values (data not shown on Figure 2) were somewhat higher

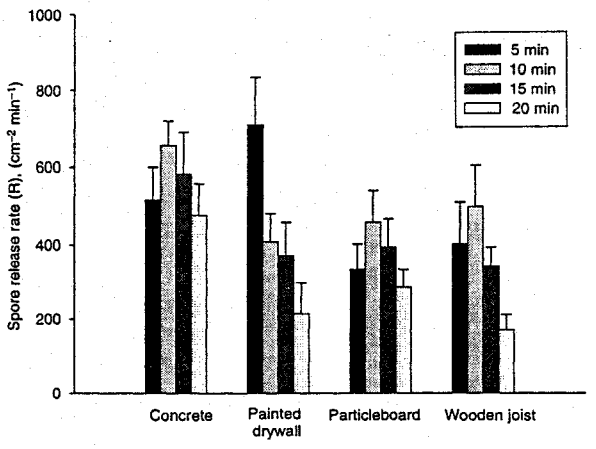

Fig. 2 Spore release rate for different contaminated surfaces and time of the FSSST application. Each R-value represents a single-test measurement and the error bars represent standard deviation of spore counts conducted on at least 20 microscopic fields

for concrete and painted dry wall than for the particleboard and wooden joist (following the initial spore surface densities, N_S), the material type had no dominant effect on the release rate. This can be attributed to the similarity in the physical properties of the four tested substrates (smooth and essentially non-porous materials). Other smooth non-porous materials, such as gypsum board release spores at about the same rate, while some porous substrates, such as ceiling tiles, may exhibit rates as high as approximately $10^4/cm^2/min$ (Sivasubramani et al., 2003). Figure 2 shows that the most intense spore release occurred during the first 10 min (concrete, particleboard, and wooden joist) or the first 5 min (painted drywall) of the 20-min FSSST application. A higher temperature and especially lower relative humidity recorded in the home with mold-contaminated drywalls suggest that fungal spores in that environment were easier to release (perhaps, in a greater extent than in other tested environments) (Foarde et al., 1999; Pasanen et al., 1991). Therefore, the majority of spores aerosolized during the first 5 min. For all the four tested materials, the spore release was observed continuously during the 20-min experiment and the release rate was significantly greater during the first 10-min than during the second 10-min of the 20-min interval. This finding confirms the results of our earlier laboratory study (Sivasubramani et al., 2003) performed with ceiling tiles, gypsum board, and agar artificially contaminated with *Aspergillus versicolor*. Similarly, Górny et al. (2001) reported that about 71–88% of the spores were released during the first 10-min of the 30 min experiment. The most matured spores are released within the first few minutes of the application of the external forces (in this case, air current) whereas longer exposure time is needed for the less matured spores.

The microscopic analysis showed that the spore types in the FSSST samples matched the ones identified in the swab samples on the respective contaminated surfaces. Interestingly, the FSSST samples revealed only the same spore types that were in the respective swab samples. This indicates that the chances of contamination of FSSST samples from the ambient air are negligible. At the same time, the ambient air samples had several spore types that were not identified in FSSST or swab samples. This demonstrates that the FSSST samples are more representative of the fungal source than the air samples taken in the vicinity of the source. In contrast to the traditional swab sampling method, the FSSST allows assessing the potential release of spores from the source in the worst-case scenario, which consequently provides the data for estimating the maximum plausible indoor concentration of spores.

Like for the total spore release, the release rate of *Aspergillus/Penicillium* spores was highest during the first 10 min for concrete, particleboard, and wooden joist, and during the first 5 min for the painted drywall. The *Cladosporium* spores were released from concrete and wooden joist primarily during the first 10-min interval. No *Cladosporium* spores were detected on the FSSST samples taken from the particleboard. The swab sample analysis also revealed the absence of *Cladosporium* spores on this surface. Few *Cladosporium* spores were found to have released from the painted drywall. *Chaetomium* spores were aerosolized from the painted drywall and particleboard primarily during the first 5–10 min. *Stachybotrys* spores were detected only on the FSSST samples taken from the painted drywall, and the release rate of this spore type showed high variability from one test to another (Figure 3). As the *Stachybotrys* is considered to be a wet spore type fungus (Duchaine and Mériaux, 2001), the local variation of the moisture level inside the fungal colony may have a great influence on the spore release into the air environment.

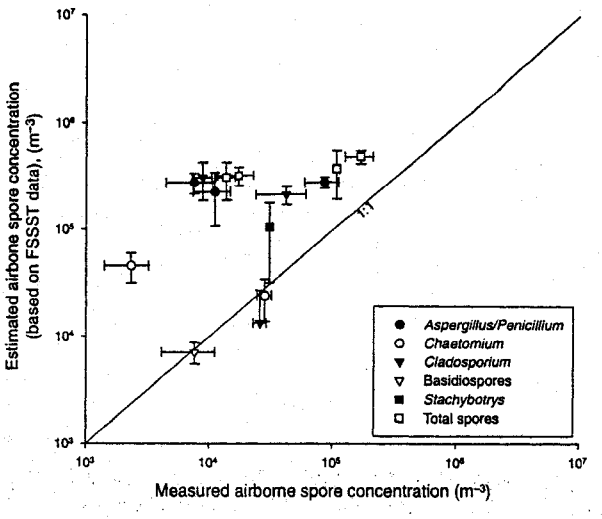

Fig. 3 The estimated airborne spore concentration vs. measured airborne spore concentration. Error bars indicate the standard deviation of four tests

Table 1 shows the relative efficiency of the spore release. This category allowed us to relate the release rate (measured from the 10-min FSSST samples) to the initial spore surface density (determined from swab samples). Consequently, we compare the spore release from different materials having different initial spore surface density. The E_R-values are listed for the total spore count and for individual spore types. The data demonstrate that only a small fraction (not exceeding several percent) of the spores present in the substrate are aerosolized by the FSSST, although it creates very favorable aerosolization conditions. This finding agrees well with the data reported in our recent laboratory studies (Sivasubramani et al., 2002, 2003), in which low E_R-values were reported for other substrates. For the total spore count, the highest release efficiency was

Table 1 Relative efficiency of spore release

Building material	Spore type	Relative efficiency (%)
Concrete	Total spores	1.56 ± 0.22
	Aspergillus/Penicillium	1.48 ± 0.17
	Cladosporium	1.68 ± 0.32
Painted drywall	Total spores	0.62 ± 0.30
	Aspergillus/Penicillium	2.30 ± 1.22
	Chaetomium	0.12 ± 0.05
	Cladosporium	0.28 ± 0.29
	Basidiospores	0.28 ± 0.06
	Stachybotrys	0.45 ± 0.31
Particleboard	Total spores	0.28 ± 0.11
	Aspergillus/Penicillium	1.51 ± 0.62
	Chaetomium	0.05 ± 0.01
Wooden joist	Total spores	0.18 ± 0.07
	Cladosporium	0.18 ± 0.07

The data shows the average and standard deviation values of four tests.

found for concrete surfaces followed by painted drywall, particleboard and wooden joist. As described above, the spore release rate did not show a considerable dependence on the substrate material, which was attributed to the similar physical properties of the test materials. Therefore, the relative efficiency of spore release was greater for the substrate that had a lower initial spore surface density. Thus, the materials are in reverse order with respect to E_R (total spores) comparing to their N_S: the highest E_R was detected for concrete and the lowest for wooden joist. For the *Aspergillus/Penicillium* group, $E_R = 2.30 \pm 1.22\%$ for the painted drywall and $1.48 \pm 0.17\%$ for concrete, and the particleboard showed only $1.51 \pm 0.62\%$. Our laboratory study (Sivasubramani et al., 2003) of *Aspergillus/Penicillium* spore release from the naturally contaminated gypsum board showed $E_R = 1.39 \pm 0.85\%$, which is comparable with the data found in this study for all three materials on which *Aspergillus/Penicillium* spore growth was detected. Generally, the E_R-values may exhibit dependence on the substrate for the same fungal species, if the physical properties (the surface smoothness, porosity, etc.) are distinctly different. For example, the relative efficiency of release of *Aspergillus versicolor* spores ranged from $0.002 \pm 0.001\%$ (for the laboratory-inoculated agar) to $9.2 \pm 1.0\%$ (for the laboratory-inoculated gypsum board) (Sivasubramani et al., 2003). The relative efficiency of release of *Cladosporium* spores was found to be higher on the concrete substrate ($1.68 \pm 0.32\%$) than on painted dry wall ($0.28 \pm 0.29\%$) and wooden joist ($0.18 \pm 0.07\%$), following the trend observed for the total spore count. Overall, the concrete surface and painted drywall provide low nutritional condition and high resistance to the fungal growth. As a result, the spores grown on these surfaces are less bounded with conidiophores (and thus ready to release) than those grown on the particleboard and wooden joist. The data show that the efficiency of spore release may vary with the fungal type, although it remained below 1% for all the identified types, except for *Aspergillus/Penicillium* and *Cladosporium*.

As the FSSST simulates the conditions most favorable for the spore aerosolization, we utilized the collected data for assessing the fungal spore concentration in indoor air, assuming that all spores were released from identified indoor sources. The assessment was conducted for a typical room with approximately 1% of the total surface area heavily contaminated with fungi. The following dimensions were applied: $V_{ROOM} = 7 \times 4 \times 4$ m $= 112$ m^3 (which is an average volume of the four test rooms selected for this study); $A_{CONTAMINATION} = 1$ m^2. The airborne spore concentration was estimated assuming that the spores were released from the growth substrate at a rate as high as the one provided by the FSSST during 10 min (t_{FSSST}) (defined as a worst-case scenario). The estimated concentrations of different fungi, identified in four homes with different mold-contaminated substrates, are plotted in Figure 3 against the airborne spore concentrations, which were actually measured in the respective homes ($C_{AIR}^{MEASURED}$). For each fungal type, the estimated values were greater than (or about the same as) the measured ones. The estimated total airborne spore concentration ranged from (31 ± 12) to $(50 \pm 7) \times 10^4$/m^3, while the actual concentration was between (1.4 ± 0.2) and $(17.1 \pm 4.3) \times 10^4$/m^3, confirming that the FSSST data may be used for the assessment of maximum potential exposure. In relative sense, the above fungal aerosol concentrations (both the estimated and measured in homes) are rather high. For example, a review article by Rao et al. (1996) referred to $C_{AIR}^{MEASURED}$ of up to approximately 10^4/m^3 for typical water-damaged buildings (this level can be considered to represent extremely contaminated indoor environments).

Conclusions

A recently-developed FSSST, which allows assessing the potential of fungal sources to aerosolize spores into indoor air, was used to investigate the spore release from four building materials in mold-problem homes. The spores of different species were efficiently aerosolized by the FSSST from these materials, exhibiting the total spore release rate ranged approximately from 10^2 to 10^3/cm^2/min. The data show that only a small fraction (not exceeding several percent) of the spores present on the surface are aerosolized by the FSSST although it creates very favorable aerosolization conditions. The relative efficiency of the total spore release was highest for concrete, followed by painted drywall, particleboard and wooden joist. The spore release efficiency was found to depend on the fungal type and growth conditions. The aerosol spore concentration estimated from the release rate data was found in most

of the cases to be greater than that actually measured in these environments with air sampling. The results suggest that the FSSST, which provides the most favorable conditions for the spore aerosolization, can be used for assessing the maximum potential exposure to airborne spores released from identified sources in homes.

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Fungal spore source strength tester: laboratory evaluation of a new concept

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Abstract

The airborne fungal spore concentration measured with air samplers during specific time intervals does not always adequately represent the maximum spore concentration levels, because of the sporadic nature of spore release. Hence, a reliable method is needed to directly assess the indoor fungal sources with respect to their spore aerosolization potential. In this study, the newly developed fungal spore source strength tester (FSSST), which aerosolizes spores from growth surfaces and samples the airborne fungi into a bioaerosol sampler, was evaluated in the laboratory. The FSSST's operational flow rates of 30 and 12.5 l/min were tested. The fungal spores released from moldy surfaces were measured with an optical particle counter. Simultaneously, the spores were collected by a bioaerosol sampler: either with a 37-mm filter cassette or with the BioSampler. Three material types, ceiling tile, gypsum board and plastic sheet coated with agar, were tested after they were inoculated with the fungus *Aspergillus versicolor*. In addition, gypsum board naturally contaminated with various fungi (obtained from a mold-problem home) was tested in the laboratory using the FSSST. In all three laboratory-inoculated materials, the release rate of *A. versicolor* was found to be higher when the FSSST operated at 30 l/min than at 12.5 l/min. Nevertheless, even at 12.5 l/min the number of spores aerosolized from the source during 10 min was found sufficient to reflect the highest level of release that may occur in indoor environments. At 12.5 l/min, the release rate of *A. versicolor* during the first 10-min period was $(23.9 \pm 17.7) \times 10^4$ cm^{-2} for ceiling tile, $(1.3 \pm 0.3) \times 10^4$ cm^{-2} for gypsum board and $(0.13 \pm 0.08) \times 10^4$ cm^{-2} for agar surface (based on the samples collected with the BioSampler). The spore release rate was higher during the first 10 min than during the second 10 min of the FSSST application. It was observed that the particles aerosolized from the *A. versicolor* culture included spore aggregates and single spores, as well as mycelial fragments. Overall, 0.6 ± 0.3% of spores detected on 1 cm^2 of ceiling tile inoculated with *A. versicolor* were aerosolized during the 10-min source testing. The respective number was 9.2 ± 1.0% for the laboratory-inoculated gypsum board, 0.002 ± 0.001% for the laboratory-inoculated plastic covered with agar and 1.8 ± 0.2% for naturally contaminated gypsum board. Our data suggest that the FSSST provides very favorable conditions for the spore aerosolization and thus can be used in the field to assess the maximum potential spore release from a fungal source.

Keywords: Bioaerosols; Fungi; Spore release; Building material; Source strength

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1. Introduction

Microbial contamination of indoor environments has gained special attention because of adverse health effects associated with bioaerosols. Human exposure to fungi has been known to cause various types of health problems, primarily irritations, infections, allergies and toxic effects. It has been suggested that toxigenic fungi may cause additional adverse health effects (Croft et al., 1986; Etzel et al., 1998; Hodgson et al., 1998; Epstein and Fan, 2001). The prevalence and severity of adverse health effects associated with fungal exposure in both children and adults have been reported to increase when visible mold growth or water damage is detected in an indoor environment (Miller, 1995; Strachan and Carey, 1995; Verhoeff et al., 1995; Hu et al., 1997; Koskinen et al., 1997; Maier et al., 1997; Verhoeff and Burge, 1997; Sigsgaard et al., 1999).

Fungi can grow on almost any building material if there is enough moisture available (Pasanen et al., 1994). Moreover, the high cellulose content in building materials such as ceiling tiles provides an ideal environment for fungal growth. Presently, it is common to construct walls, both interior and exterior, with wall systems that use gypsum wallboard, ceiling tiles, fiberglass insulation and vinyl wallpaper. These building materials contain nutrients, which are available for fungal colonization if adequate moisture is present. North American cross-sectional questionnaire studies have revealed that 27–36% of homes exhibit mold problems (Dales et al., 1991; Spengler et al., 1993). A Finnish study conducted by Nevalainen et al. (1998) showed that 80% of 450 randomly chosen houses of different age were affected by moisture problem.

The formation and emission of the fungal spores from their sources are affected by water activity, humidity condition in the environment, air temperature, availability of oxygen, presence of nutritional sources, electrostatic and ionic interactions and the texture of the contaminated surface (Becker, 1994). Kildesø et al. (2003) studied the release of spores of nine typical indoor fungi from wet wallpapered gypsum board under controlled conditions and found that the spore release rate significantly differs for different fungi under identical conditions. They also found that the relationship between the air velocity and the spore release rate depends on the fungal type.

The data on the fungal spore aerosolization from agar and ceiling tiles were reported by this research group in the publication of Górny et al. (2001). This study revealed that the parameters such as fungal species, air velocity above the surface, texture of the surface and vibration of the contaminated material, affected the fungal spore release. In a more recent paper, Górny et al. (2003) reported that relatively gentle air currents can release bacterial spores of *Streptomyces albus* along with cell fragments. The spore aerosolization from growth surfaces is driven by energy from external sources, as well as by various environmental factors. Spore release by air currents is likely the most prevalent mechanism for indoor fungi (Gregory, 1973; Madelin, 1994). The amount of fungal spores released from agar and fiberglass duct material was found to be proportional to the air velocity and inversely proportional to the air humidity (Zoberi, 1961; Pasanen et al., 1991; Foarde et al., 1999). Duchaine and Mériaux (2001) pointed out that dry spores such as *Penicillium* sp., *Aspergillus* sp. and *Cladosporium* sp. can be easily released into air, while wet spores such as *Stachybotrys* sp., *Acremonium* sp. and *Trichoderma* sp. are not always easily aerosolized. Górny et al. (2001) concluded that turbulent air flow can liberate *Aspergillus* and *Penicillium* spores more easily than *Cladosporium* spores. This is because the first two types of fungi have the most mature spores naturally located above the mycelial surface, while for the last one these spores are imbedded in the hyphal structure.

The airborne fungal spore concentration measured with bioaerosol samplers during specific time intervals may not be representative of indoor air quality. While numerous aerosol samplers are available (Muilenberg, 2003), an assessment method based on the conventional air sampling of spores may not be able to reliably detect fungal colonization on indoor surfaces and thus may only approximate potential exposure level (Horner, 2003). In many situations, indoor air sampling has not been sufficient in identifying mold problems.

in housing or establishing a relationship between airborne mold concentration and health effects. In part, this is because the spore release does not necessarily occur during the air sampling. Since the fungal spore emission is rather irregular and sporadic and as it is affected by variations in physical conditions, fungal spores may not be well mixed in the environment. As a consequence, short-term air sampling data do not always adequately represent the highest possible spore concentration levels that may occur in indoor environments. Hyvärinen et al. (2001) found that the fungal spore concentration in residential indoor environments may vary significantly within a day. Buttner and Stetzenbach (1993) conducted a study in an experimental room and concluded that in certain situations, air sampling alone may not adequately reflect the level of microbial contamination of indoor environments. Although most of the health effects are associated with airborne fungi, many currently available techniques (such as bulk sampling, surface sampling and dust sampling) assess the source, but not the air contamination. Furthermore, in a number of cases, no strong association has been established between the bulk/surface/dust and airborne sampling data collected in mold-problem buildings. Chew et al. (2003) reported that the dustborne and airborne fungal propagules represent a different spectrum of fungi in a residential environment. Thus, the conventional techniques may be inadequate for assessing the potential bioaerosol exposure of the occupants.

It has been widely recognized that a reliable method is needed to directly assess the aerosolization potential of indoor fungal sources. Such a method is to be used in conjunction with conventional air sampling methods in indoor environments. In order to estimate the 'worst case scenario' of air contamination with spores aerosolized from indoor surfaces, it is important to assess the fungal source under the conditions most favorable for aerosolization. We have developed a new concept and a device called the fungal spore source strength tester (FSSST) for assessing the potential of fungal sources to aerosolize spores (Grinshpun et al., 2002a). In the present study, we have evaluated the new concept and the prototype device using different contaminated materials.

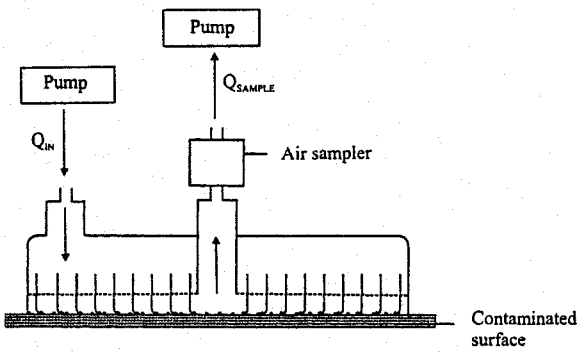

Fig. 1. Fungal spore source strength tester.

The design of the FSSST prototype is shown in Fig. 1. The tester is a cup-like device with a square external cross-section of 18.1×18.1 cm, which is held against the mold-contaminated surface. The spores are released from the surface by small air jets originated in 112 orifices (0.4 mm diameter), which are distributed over an internal cross-section of 9.5×9.5 cm, and directed towards the source. The aerosolized spores are then collected into an air sampler, attached to the tester and operated at a flow rate of Q_{SAMPLE}. The incoming flow rate, Q_{IN}, is slightly lower than Q_{SAMPLE} to create a negative pressure inside the cup. This difference prevents contaminating the ambient air with the aerosolized spores.

2. Materials and methods

2.1. Preparation of test materials

The fungal species of *Aspergillus versicolor* was inoculated on the tested building materials in the laboratory condition. This fungus is a common indoor contaminant and one of the indicator organisms for mold problems in indoor environments. Three types of materials were inoculated with *A. versicolor*:

- ceiling tiles (Armstrong World Industries, Lancaster, PA), as this material provides considerable subsurface for mold growth (due to its porous structure);

- gypsum board (National Gypsum Company, Buffalo, NY), as it represents a material with rather high potential of the spore release (due to its smooth and dry surface); and
- a plastic sheet coated with wet malt extract agar (Becton Dickinson Microbiology Systems, Sparks, MD) as it represents a medium, which provides the least favorable condition for the spore aerosolization (this sticky wet material simulates a damp surface area usually observed in flooded homes).

The identical round pieces of tested materials, with a diameter of 8.7 cm (surface area = 59.42 cm^2) and a thickness of 1.4 cm, were prepared and sterilized before the experiments. The *A. versicolor* strain was first grown on malt extract agar. The spore suspensions were prepared from the well-grown culture, and 1 ml of the suspension was inoculated onto each test material. The detailed description of the inoculation and incubation procedures has been presented in Górny et al. (2001).

In addition to the tests conducted with laboratory-inoculated materials, naturally contaminated gypsum board was also used to study the spore aerosolization. The gypsum board was brought from a moldy home into the laboratory and tested similarly to the three artificially contaminated materials. Prior to the experiment, the naturally contaminated gypsum board was placed in a clean box and dried at a room temperature to achieve the same surface moisture level as that of the laboratory inoculated materials (25–32%).

2.2. Characterization of test materials with respect to the initial spore contamination on the surface

Prior to the experiment, the test surfaces were characterized with respect to the initial spore contaminations using a bulk sampling method. A sample of approximately 1 cm^2 was cut from the 59.42 cm^2 piece of the test material and suspended in 20 ml de-ionized, sterilized water with 0.05% Tween 80 (Sigma Chemicals Co., St. Louis, MO) in a test tube. The spores were then extracted from the material sample using a vortex touch mixer (model 231, Fisher Scientific Company, Pittsburgh, PA). A 3 ml aliquot of spore suspension was taken from the 20 ml stock, and filtered through a 13-mm mixed cellulose ester filter (0.8 μm pore size) by using a analytical stainless-steel vacuum filter holder (Fisher Scientific Company, Pittsburgh, PA). The filters were then air dried in sterile Petri plates at room temperature for 2 h. The dried filters with the spores extracted from the material sample were placed on a glass slide and cleared by acetone vapor utilizing a modified instant acetone-vaporizing unit (model Quickfix, Environmental Monitoring Systems, Charleston, SC). A 25×25 mm cover glass was mounted on the slide using glycerin jelly (gelatin: 20 g, phenol crystals: 2.4 g, glycerol: 60 ml, water: 70 ml). The spores were counted by using a light microscope (model Leitz Laborlux S, Leica Mikroskopie und Systeme GmbH, Germany, available through W. Nuhsbaum, Inc., IL) at 400× magnification. Based on the number of spores counted on the 1-cm^2 area of the material sample, the spore surface density, N_S, was determined. The material characterization test was performed in three replicates.

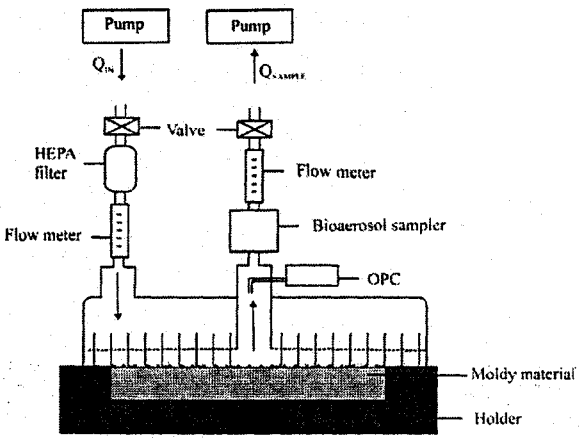

Fig. 2. Experimental setup.

2.3. Experimental design

Fig. 2 shows the experimental setup. The FSSST utilizes two vacuum pumps (push and pull). The push pump upstream of the cup creates an incoming airflow through the HEPA-filter (1244 HEPA capsule filter, PALL Gelman Laboratory, Ann

Arbor, MI). The filtered flow penetrating through the 112-orifice stage at the bottom of the device creates air jets orienting towards the surface, by which the spores are aerosolized from the moldy material contained inside the holder. The airborne spores are collected into a bioaerosol sampler. In this experimental setup, the released particles were also counted by a real time optical particle counter (OPC, Model 1.108, Grimm Technologies, Douglasville, GA), situated at the outlet tube that connects to the downstream pull pump. The flow rate balance is set so that the downstream airflow (Q_{SAMPLE}) rate is higher than the upstream air (Q_{IN}) by $\Delta = 0.5$ l/min. The airflow rates are controlled at the FSSST's inlet and outlet with two flow meters (model 2A17, Key Instruments, Trevose, PA) calibrated with a Buck calibrator (A.P. Buck, Orlando, FL).

Two bioaerosol samplers were used in this study: either the open-face filter holder with a 37-mm mixed cellulose ester filter (0.8 μm diameter pore size; Millipore Corp., Bedford, MA) or the BioSampler (SKC Inc., Eighty Four, PA). The filter sampler operated in one of two regimes, at 30 l/min and 12.5 l/min, respectively, as two prototypes of the FSSST were originally built (with different air jet velocities). The filters containing collected spores were placed on a glass slide and cleared by acetone vapor for microscopic counting, using the same procedure as for the material samples. Two types of spore counts were performed for the filters: propagule count (combined number of singlet and aggregates) and single spore count (each spore was counted separately, ~~weather~~ it occurred in a singlet or in an aggregate). The BioSampler operated at its nominal flow rate of 12.5 l/min with a 20 ml suspension of de-ionized, sterilized water mixed with 0.05% Tween 80. After the sampling, the suspensions were filtered through mixed cellulose ester filters, which were then cleared by acetone vapor and analyzed under the microscope.

The temperature and relative humidity, monitored with a traceable humidity/temperature pen (Fisher Scientific Company, Pittsburgh, PA), were 20 °C–24 °C and 32%–40%, respectively. The low humidity was chosen for this study to represent the 'worst-case scenario', it has been shown that fungal spores release into dry air easier than into humid air (Pasanen et al., 1991; Foarde et al., 1999). As the optical diameter of the *A. versicolor* spores was above 1.6 μm (Górny et al., 2001) this particle size was chosen as the lower counting limit for the particle-size selective OPC. The entire setup was placed inside a Class II biosafety cabinet (SterilchemGARD, Baker Company, Stanford, ME).

The two sampling flow rates of 30 and 12.5 l/min resulted in air velocities through the jet orifices of 35.5 m/s and 14.8 m/s, respectively. The duration of each separate experiment was 20 min, including two sets: the first 10 min and the second 10 min. The 10-min increments were chosen based on our previous findings (Górny et al., 2001) that the majority of spores aerosolized from the moldy surface during the first 10 min of its exposure to air jets. During both time intervals, 10 and 20 min, the FSSST was held against the same moldy surface (each time with a new filter or a replenished BioSampler collection fluid). Prior to every experiment, the system was operated in the absence of any test material in the chamber until essentially a zero-particle level was achieved, as measured by the OPC. Next, the FSSST was applied to a sterile non-incubated material to establish the background level for particles released from the test surface when exposed to airflow. The background numbers were subtracted from the number of particles measured in experiments performed with contaminated fungal surfaces. The latter was determined from the OPC counts of particles released from the contaminated surfaces.

For each set of experimental conditions, the spore release data were collected with the FSSST in three replicates. The results were expressed as the release rate (R, cm^{-2} min^{-1}) and the relative efficiency of spore release (E_R, %). The release rate is the number of airborne spores aerosolized from 1 cm^2 of the test surface during 1 min of the FSSST application. The relative efficiency of the spore release (in percent) is the number of spores released from 1 cm^2 during the time of the FSSST's application (t) to the initial number of spores on the area of 1 cm^2 determined by bulk sampling method:

$$E_R(t) = \frac{Rt}{N_S} \times 100\%$$

The average values of R and E_R and the standard deviations were calculated for each set of conditions. The data were statistically analyzed by paired t-test using software package SPSS 11.0 for Microsoft Windows.

3. Results and discussion

The initial spore surface density determined by the bulk sampling method was $(52.1 \pm 23.5) \times 10^6$ cm^{-2} for the plastic covered with agar and $(36.3 \pm 11.2) \times 10^6$ cm^{-2} for ceiling tiles. These levels were considerably higher than the one obtained with gypsum board [$(0.141 \pm 0.042) \times 10^6$ cm^{-2}]. The nutritional conditions on the agar surface and the subsurface conditions in the ceiling tile favors better growth and greater spore production than on the flat gypsum board surface with limited amount of nutrition.

To determine the airflow rate suitable to release substantial number of spores, the FSSST was tested at $Q_{IN} \approx 30$ and 12.5 l/min. At 30 l/min, for all three materials inoculated with *A. versicolor*, the particle release rate measured by the OPC was higher during the first 10 min than the following 10 min. The number of particles released during the first 10-min was in a range of $(39–70) \times 10^4$ cm^{-2} for ceiling tile, $(22–31) \times 10^4$ cm^{-2} for gypsum board and $(7–10) \times 10^4$ particle cm^{-2} for agar, respectively. Górny et al. (2001) also found the spore release from ceiling tiles to be higher than that from agar surface. When the aerosolized particles were collected on a filter at the airflow rate of ≈30 l/min, the filter was quickly overloaded with spores, which limited the accuracy of the spore enumeration by microscopic methods. Hence, it was concluded that the microscopic count could not be performed at this flow rate from most of filter samples collected with the FSSST. The use of glass slide impactors, such as the Air-O-Cell (Zefon Analytical Instruments, St. Petersburg, FL) and Cyclex-D (EMSL Analytical, Inc., Westmont, NJ), with the FSSST as an alternative to the filter was not a solution. The particle surface density on the slide deposit area was too high for an adequate microscopic count given very high spore concentration levels inside the FSSST and a small area of deposit [approx. 30 mm^2 for Air-O-Cell cassette (Aizenberg et al., 2000) and approximately 19 mm^2 for the Cyclex-D (Grinshpun et al., 2002b)].

The FSSST was also evaluated at 12.5 l/min because this flow rate is used in commercially available bioaerosol samplers such as AGI-30 (Ace Glass Inc., Vineland, NJ) and the BioSampler (SKC Inc.). Based on the OPC data, the particle release rate was found to be higher at 30 l/min than at 12.5 l/min. The increased release of particles with the increase of air velocity has also been reported by Zoberi (1961), Gregory and Lacey (1963) and Górny et al. (2001). Fig. 3 shows the particle release rate at 12.5 l/min as measured by the Grimm OPC. The number of particles released during first 10-min interval was $(9.4 \pm 4.7) \times 10^4$ cm^{-2} for ceiling tile, $(5.6 \pm 1.3) \times 10^4$ cm^{-2} for gypsum board and $(0.11 \pm 0.07) \times 10^4$ cm^{-2} for agar (see 'OPC count' in Fig. 3). The release rate was higher during the first 10 min than during the second 10 min of the experiment for all three tested materials. This finding agrees with our earlier study, which showed that 71–88% of spores were released during the first 10 min of the 30-min experiment (Górny et al., 2001).

The above OPC data were utilized to estimate the airborne spore concentration in a typical room, which would result from the spore release from contaminated surfaces at the same rate as provided by the FSSST (the worst case scenario). The aerosol concentration in a room of $6 \times 5 \times 3$ m (= 90 m^3) was estimated assuming that 1 m^2 (approx. 1%) of the surfaces were heavily contaminated with fungi and that the environmental conditions were most favorable for spore aerosolization (i.e. the release rate was assumed to be equal to the one measured with the FSSST during the first 10 min). Based on our experimental data obtained with ceiling tiles, we found that the airborne spore concentration would range from 6×10^6 to 16×10^6 m^{-3}. For gypsum board, it would range from 4×10^6 to 7×10^6 m^{-3} and for agar from 1×10^5 to 3×10^5 m^{-3}. These concentration levels are of about the same order of magnitude as those

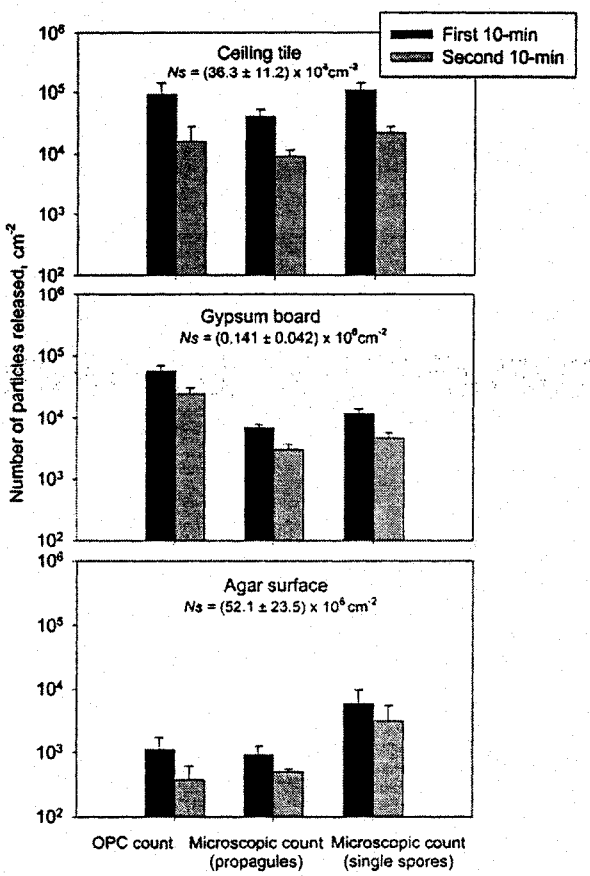

Fig. 3. The release rate of *A. versicolor* spores determined with the Grimm OPC and by the microscopic counts of propagules and single spores on the collection filters. The FSSST airflow rate was 12.5 l/min. The error bars indicate the standard deviation of three repeats. N_S = initial spore surface density.

measured by Rautiala et al. (1996) during the demolition and repair of moldy buildings, which produce rather excessive airborne spore concentration levels that can be regarded as maximum plausible. Thus, the FSSST operating at 12.5 l/min appears to be suitable for assessing the maximum potential of the fungal spore release from growth surfaces in indoor air.

When testing the FSSST at the flow rate of 12.5 l/min, the spore deposits on the collection filters were countable (in contrast to the tests conducted at 30 l/min). The microscopic enumeration was performed separately for spore propagules and single spores (see Fig. 3). The data obtained from both the propagule and single spore filter counts confirmed that almost of the spores were released during the first 10 min of the 20 min interval. Based on the single spore count, the release rate was the highest for ceiling tile [$(10.9\pm3.6)\times10^3$ cm^{-2} min^{-1}], decreasing for gypsum board [$(1.9\pm0.2\times10^3$ cm^{-2} min^{-1}] and further for agar [$(0.6\pm0.4)\times10^3$ cm^{-2} min^{-1}]. This may be due to the difference in the spore surface density in the test materials and release of agglomerates. In the case of the agar surface, the binding forces between the spores and conidiophores may restrict the release of spores, particularly for fresh cultures.

As seen from Fig. 3, for ceiling tiles, the OPC airborne particle counts were somewhat higher than the microscopic counts of propagules and about the same as the single spore counts. The OPC counts an aggregate of spores as a single particle. The aggregate may break into several smaller aggregates or single spores due to their impaction on the filter thus increasing the microscopic counts vs. the airborne OPC count. The difference between the OPC count and the microscopic count of fungal propagules on the filter was rather low suggesting that the aerosolization of particles other than spores (e.g. mycelial fragments) is not of higher efficiency in the case of the ceiling tile. The single spore count is about twice greater in average than the propagule count, which suggests that a significant number of spores were released from the ceiling tile in aggregates ($^{**}P<0.01$). In the test conducted with gypsum board, the OPC exhibited higher counts than both the propagule and single spore counts. The flat surface of the gypsum board favors the release of other particles (such as mycelial fragments), thus affecting the OPC counts. Small differences between the release rates obtained from the propagule and the single spore counts demonstrates that some spores were aerosolized from the gypsum board in aggregates ($^{*}P<0.05$). In the case of agar, the OPC count was about the same as the propagule count but lower than the single spore count. As the surface moisture content is particularly high on the agar surface, the release of hyphal fragments is anticipated to be low, thus making the OPC particle count an adequate measure of the airborne propagule concentration. At the same

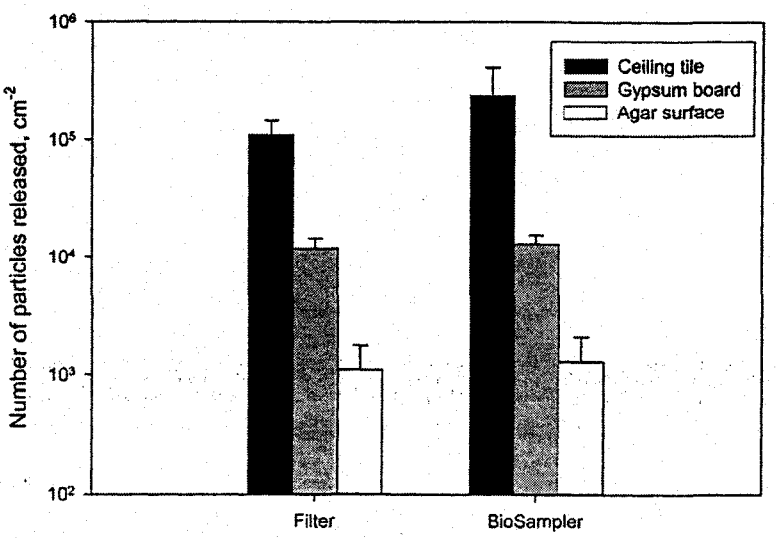

Fig. 4. The release rate of *A. versicolor* spores determined with the filter and the BioSampler operated at 12.5 l/min during the first 10 min of the 20-min experiment. The error bars indicate the standard deviation of three repeats.

time, the binding forces between the spores and the conidiophores are very high for agar, which results in a relatively low spore release rate (as compared to ceiling tile and gypsum board) and a high percentage of the released aggregates.

Overall, the spores are believed to be more easily released during the first minutes of the FSSST's application. These are primarily mature dry spores with weaker binding forces, which are located on the top of the *A. versicolor* spore chain. In contrast, immature spores are characterized by greater binding forces with conidiophores, suggesting that a longer exposure to air currents is needed for their aerosolization.

The FSSST was also evaluated with the BioSampler so that the released spores were collected into a liquid as an alternative to their collection on a filter. The collection into a liquid allows minimizing the agglomeration effect and allows analyzing the liquid samples by different methods (including the enumeration of culturabe fungi, if necessary).

Fig. 4 shows the comparative data obtained with the filter sampler and the BioSampler, when both operated at 12.5 l/min (based on the single spore count). For each of the tested materials, the mean values were slightly greater for the BioSampler than for the filter but this difference was not statistically significant ($P>0.05$). This likely reflects somewhat more efficient spore deaggregation that occurs in the BioSampler as compared to the filter. The BioSampler data demonstrated that $(23.9\pm17.7)\times10^4$ cm^{-2} *A. versicolor* spores were released from the ceiling tile during the first 10 min, while $(1.3\pm0.3)\times10^4$ cm^{-2} were released from the gypsum board and $(0.13\pm0.08)\times10^4$ cm^{-2} from agar during the same time.

Fig. 5 presents the relative efficiency of the *A. versicolor* release during the first 10-min interval of the FSSST application (based on the single spore count obtained from samples collected with the BioSampler). As the 10-min time was found to be sufficient to assess the fungal source aerosolization potential, $t=10$ min was used to determine E_R. The gypsum board showed higher relative efficiency of spore release ($9.2\pm1.0\%$) followed by ceiling tile ($0.6\pm0.3\%$) and plastic coated with agar ($0.002\pm0.001\%$). This may be due to the nature of the gypsum board, which is often flat, non-porous and dry. The ceiling tile has a porous structure, which may trap aerosolized spores inside the pores. Thus, some spores might have not been released from the tile and detected by the bioaerosol sampler. In other words, even if some spores

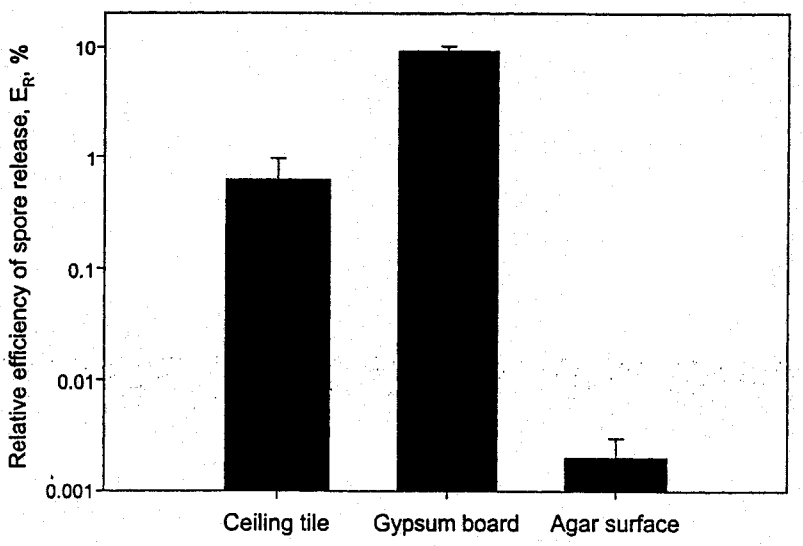

Fig. 5. The relative efficiency of *A. versicolor* spore release during the first 10-min FSSST application (based on the data obtained with BioSampler). The error bars indicate the standard deviation of three repeats.

became airborne inside the pore-pockets, the airflow energy may not have been sufficient to release them out of the pockets. The agar surface had higher moisture content, and hence, the binding force may be much greater than in the two other building materials, resulting in a lower release of *A. versicolor* spores. For comparison, Kildesø et al. (2002) reported the percentage of spores of *Penicillium chrysogenum* and *Trichoderma harzianum* released from wet gypsum boards ranging from 0.8% to 6.7% and 0.02% to 1.02%, respectively, depending on the age of the fungal growth.

The tests conducted with the gypsum board (obtained from mold-problem building and naturally contaminated with various fungal species) revealed three types of fungal spores, *Aspergillus/Penicillium*, *Chaetomium* and *Cladosporium*, as was detected from the material sample. In addition, some unidentified spores (those which could not be identified by our analysis) were found in the material sample. The spores aerosolized from the naturally contaminated gypsum board surface were collected into the BioSampler. *Aspergillus/Penicillium*, *Chaetomium*, *Cladosporium*, and unidentified spores were detected in the BioSampler collection fluid, as they became aerosolized from the board. Again, the total number of released spores observed during the first 10-min interval $[(22.2\pm8.9)\times10^3\ cm^{-2}]$ was higher than that of the second 10-min interval $[(8.7\pm2.9)\times10^3\ cm^{-2}]$. The real-time measurement data provided by the Grimm OPC confirmed this finding. The individual spore types also demonstrated similar release patterns. Fig. 6 shows the relative efficiency of spore release for total count and for individual spore types. The aerosolization data are based on the microscopic enumeration of single spores. The efficiency of total spore release from the gypsum board was rather low ($1.8\pm0.19\%$) (see Fig. 6) compared to the value obtained using the same material artificially inoculated with *A. versicolor* ($9.2\pm1.0\%$) (see Fig. 5). This difference demonstrates that the spore release rate depends on the fungal type so that different fungi may release very different quantities of spores under identical conditions as pointed out by Kildesø et al. (2003). The efficiency of *Aspergillus/Penicillium* spore release from the naturally contaminated gypsum board was $1.39\pm0.85\%$, which is also lower than was found for the same material, artificially inoculated with *A. versicolor*. The interaction of different fungi can cause hyphal net-

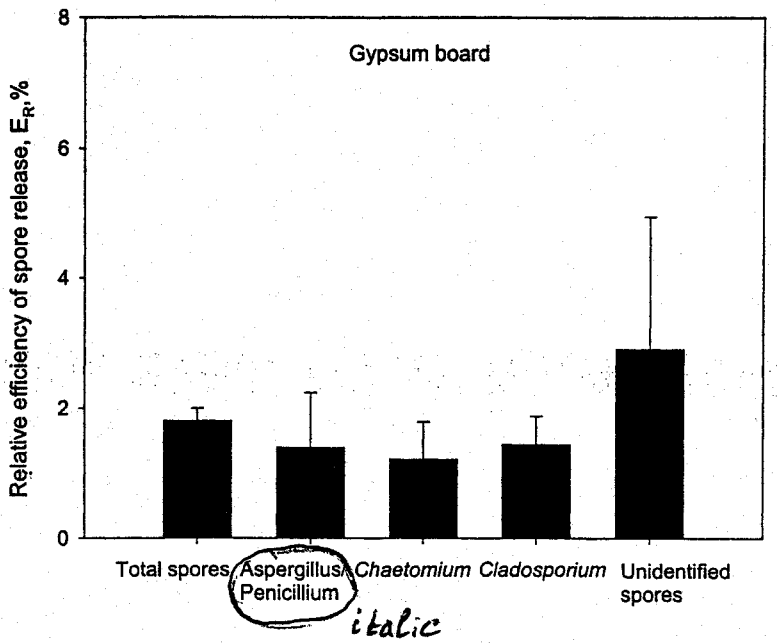

Fig. 6. The relative efficiency of spore release during the first 10-min FSSST application to the naturally contaminated gypsum board obtained from a mold-problem home (based on the BioSampler data). The error bar indicates standard deviation of three repeats.

working as well as the development of mycelial flush and byproducts on the growth surfaces (White et al., 1998). Thus the efficiency of spore release may be affected by the interaction among different fungal types growing together in case the material is contaminated with a variety of species. Therefore, the release rate of a specific fungus may be different, depending whether it has grown on a material as a single contaminant or together with other types of fungi.

4. Conclusion

The new source assessment concept is believed to be useful to assess the highest possible level of spore concentration, which may occur in an indoor environment, by measuring the aerosolization potential in the vicinity of a contaminated surface. This concept was incorporated into a newly-developed fungal spore source strength testing method that can supplement existing air quality assessment methods for more accurate exposure assessment in mold-problem buildings. The FSSST prototype was evaluated under the laboratory condition with respect to its ability to aerosolize spores from moldy surfaces and measure them. As a result, the FSSST appeared to be a promising tool for assessing the potential release of fungal spores from mold-contaminated surfaces.

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