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Aerodynamic characteristics and respiratory deposition of fungal fragments

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Abstract

The purpose of this study was to investigate the aerodynamic characteristics of fungal fragments and to estimate their respiratory deposition. Fragments and spores of three different fungal species (*Aspergillus versicolor*, *Penicillium melinii*, and *Stachybotrys chartarum*) were aerosolized by the fungal spore source strength tester (FSSST). An electrical low-pressure impactor (ELPI) measured the size distribution in real-time and collected the aerosolized fungal particles simultaneously onto 12 impactor stages in the size range of 0.3–10 μm utilizing water-soluble ZEF-X10 coating of the impaction stages to prevent spore bounce. For *S. chartarum*, the average concentration of released fungal fragments was 380 particles cm^{-3}, which was about 514 times higher than that of spores. *A. versicolor* was found to release comparable amount of spores and fragments. Microscopic analysis confirmed that *S. chartarum* and *A. versicolor* did not show any significant spore bounce, whereas the size distribution of *P. melinii* fragments was masked by spore bounce. Respiratory deposition was calculated using a computer-based model, LUDEP 2.07, for an adult male and a 3-month-old infant utilizing the database on the concentration and size distribution of *S. chartarum* and *A. versicolor* aerosols measured by the ELPI. Total deposition fractions for fragments and spores were 27–46% and 84–95%, respectively, showing slightly higher values in an infant than in an adult. For *S. chartarum*, fragments demonstrated 230–250 fold higher respiratory deposition than spores, while the number of deposited fragments and spores of *A. versicolor* were comparable. It was revealed that the deposition ratio (the number of deposited fragments divided by that of deposited spores) in the lower airways for an infant was 4–5 times higher than that for an adult. As fungal fragments have been shown to contain mycotoxins and antigens, further exposure assessment should include the measurement of fungal fragments for evaluating mold exposures in damp buildings.

Keywords: Fragment; Spore; Mold; Exposure; Electrical low-pressure impactor

1. Introduction

Fungi can grow on almost any building material if there is enough moisture available. Therefore, water

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damage in buildings is often associated with mold problems. Epidemiological studies have shown that people living or working in mold problem buildings exhibit more respiratory symptoms and diseases than people in non-problem buildings (Husman, 1996; Dearborn et al., 1999; Etzel et al., 1998; Meklin et al., 2002). The association between mold exposure and irritative symptoms of the respiratory tract and eyes is strong. The relative risk of allergic diseases in association with water-damaged houses was found to be two- to three-fold greater in various studies (Waegemaekers et al., 1989; Andrae et al., 1988; Platt et al., 1989; Dales et al., 1991; Dekker et al., 1991; Kilpeläinen et al., 2001). Both increased risk of asthma and an increase in asthmatic symptoms have been found to be associated with mold exposures or damp houses (Husman, 1996). However, a cause-and-effect relationship has not been well established with a fungal spore-targeted exposure assessment.

Several field studies have shown that the concentration of airborne fungal spores in mold problem buildings is not necessarily higher than in non-problem buildings. In a Finnish study, the mean fungal spore level in the samples collected in moldy homes was even lower than that in the reference homes (Nevalainen et al., 1991). A British study could not find a significant difference between median counts of colony forming units obtained in moldy and non-moldy homes, respectively (Strachan et al., 1990). Similarly, studies performed in Australian and the Boston area homes did not show a significant association between evidence of dampness or visible mold and the spore concentration level (Garrett et al., 1998; Chew et al., 2003).

The fungus *Stachybotrys chartarum* is suspected to be an important contributor to adverse health effects, particularly pulmonary hemorrhage in infants (Etzel et al., 1998). Its spores are produced in slimy heads and are difficult to be aerosolized (Anderson and Nissen, 2000). The aerodynamic size of *Stachybotrys* spores has been reported to be 4.2–4.6 μm (Sorenson et al., 1987; Reponen, 1995), which is considered to be too large to stay airborne for a long time and to penetrate deep into the human lung. Even in moldy buildings, concentrations of *S. chartarum* have been very low compared to the more common *Penicillium* and *Aspergillus* species. In southern California, *Stachybotrys* was found in the air of 2.9% of homes of allergic patients, and the mean concentration of culturable fungi was only 0.3 CFU m^{-3} (Kozak et al., 1980). Out of 1717 buildings across the US, it was identified only in 6% of buildings with the mean concentration of 12 CFU m^{-3} (if it was present), and only 2 out of 45 homes that reported health complaints were positive for *Stachybotrys* (Shelton et al., 2002). In a Cleveland study of severe acute pulmonary hemorrhage in infants, only 43 CFU m^{-3} of *S. chartarum* were measured in homes compared to the total fungi concentration of 29,227 CFU m^{-3}, and no *S. chartarum* spores were measured in 4 out of 10 homes where patients lived (Etzel et al., 1998). This suggests that other types of fungal components should be explored when performing the fungal exposure assessment.

Recent epidemiological studies have reported strong relationship between fine particles and adverse health outcomes. Even low concentrations of particulate air pollution can cause adverse health effects, and fine particles (<2.5 μm) were found to be significantly associated with elevated mortality and morbidity risk, especially from cardiovascular diseases and respiratory causes (Dockery et al., 1993; Magari et al., 2001 and 2002; Pope, 2000; Pope et al., 1999; Gold et al., 2000; Pekkanen et al., 2002). These results galvanized an interest to investigating the possible role of fine particles in fungal exposures. In our recent laboratory studies (Górny et al., 2002, 2003), we found that large quantities of smaller-sized fungal and actinomycete fragments are released together with spores from contaminated surfaces. These studies demonstrated that the number of released fragments was always higher than the number of intact spores released from contaminated surfaces. The trend does not seem to be dependent on the air velocity, the surface material, or vibration applied to the surface. Fungal fragments have been shown to contain fungal antigens (Górny et al., 2002) and mycotoxins (Brasel et al., 2005). This suggests that fungal fragments may potentially contribute to the adverse health effects and raises the need for further characterizations of fungal fragments with respect to their aerodynamic behavior.

In the present study, the aerodynamic characteristics of three different types of fungal particles released from contaminated surfaces under controlled laboratory conditions were analyzed using an electrical low-pressure impactor (ELPI; Dekati Ltd., Tampere, Finland). To achieve this goal, three different sampling conditions were tested for their effectiveness in preventing spore bounce in the ELPI. Based on the size distribution data on the airborne fungal particles obtained from the experiments, the respiratory deposition of fungal particles was calculated and compared between fragments and spores.

2. Materials and methods

2.1. Test microorganisms and their preparation for measurements

This study was conducted with three different species of fungi: *Aspergillus versicolor*, *Penicillium melinii*, and a non-toxic strain of *S. chartarum*. *A. versicolor* and *S. chartarum* are among the species that are commonly found in moldy materials (Institute of Medicine, 2004).

Penicillium species are common both in non-problem and problem buildings (Meklin et al., 2002; Shelton et al., 2002). These species were selected to represent different aerodynamic sizes of fungal spores: 2.5 μm (*A. versicolor*), 3.0 μm (*P. melinii*) and 5 μm (*S. chartarum*) (Reponen, 1995; Reponen et al., 1996, 2001; Sorenson et al., 1987). *A. versicolor* and *P. melinii* are the same strains used in our previous studies (e.g., Reponen et al., 2001; Górny et al., 2002). The non-toxic isolate of *S. chartarum* was isolated from an environmental sample and characterized as isolate JS5105 by Jarvis et al. (1998). All fungal species were grown on malt extract agar (MEA) plates at 24 °C and a relative humidity of 97–99% for one month to obtain abundant fungal growth.

2.2. Aerosolization and analysis of the size distribution of fungal particles

The experimental setup used in this study is schematically shown in Fig. 1. It consists of two parts, one for the aerosolization and the other for the collection and quantification of fungal particles. Fungal aerosolization was conducted by a recently developed fungal spore source strength tester (FSSST), which has been designed to aerosolize fungal particles from contaminated surfaces by high-speed jet air using HEPA-filtered air (Sivasubramani et al., 2004a, b). The collection and quantification was performed using the ELPI. It is a real-time particle size analyzer consisting of a corona aerosol charger, real-time multi-channel electrometers measuring real-time particle concentrations, and a low-pressure cascade impactor classifying and collecting particles into 12 size fractions within the size range of 0.03–10 μm. A corona charger of the ELPI charges incoming particles positively, and the charged particles induce positive current on the impactor stages at the time of the collection. Then, the electrometers in the ELPI measure currents on the impactor stages and the data processor of the instrument converts them to the particle number concentration. The entire set-up was placed inside a Class II biosafety cabinet (SterilchemGard; Baker Company, Inc., Sanford, ME). The exit flow from the ELPI was filtered with a HEPA-filter (12144 HEPA capsule filter; Pall Corporation, Ann Arbor, MI) and redirected into the biosafety cabinet to prevent contamination of the room environment. The aerosol background level was measured before starting the aerosolization tests by placing an open agar plate without fungal growth in the FSSST. The background concentrations were found to be negligibly low. After 1 month of incubation, fungal particles were released from a sporulating agar plate by operating the FSSST for 5 min at a flow rate of 30 l min^{-1}. The aerosolization of fungal particles was repeated three times for each fungal species. The ELPI quantified the concentration of collected fungal particles on the 12 impactor stages.

2.3. Testing the three methods for preventing spore bounce inside the ELPI

During the pilot operation of the experimental setup with *A. versicolor*, a negative value of current in the ELPI was observed in the particle size range of 0.1–1 μm. This was attributed to bounce-off of fungal spores from the stages above this size range. The particle bounce and reentrainment is one of the limitations of an inertial aerosol impactor. In order to minimize the particle bounce problem, a sticky material has been traditionally used for coating the impaction plates.

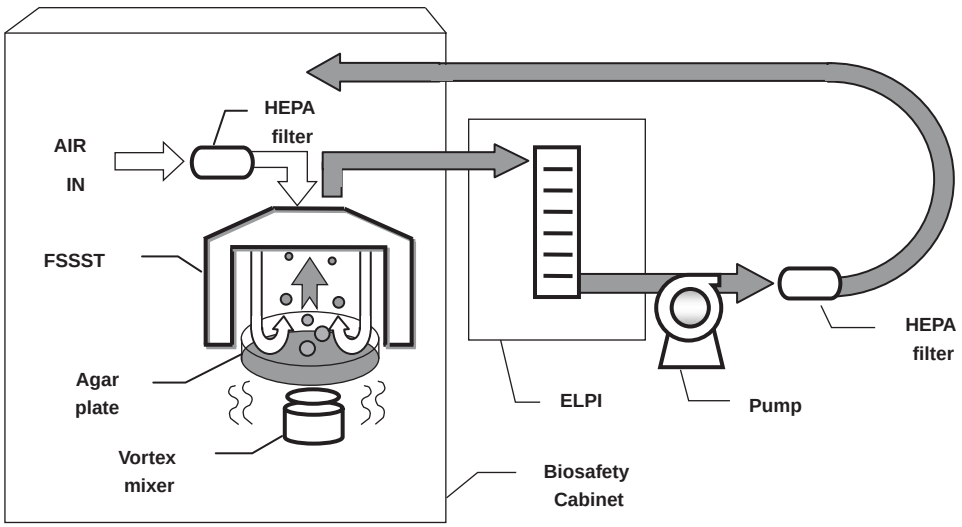

Fig. 1. Experimental set-up for the release and collection of fungal particles.

However, when utilizing certain analytical techniques, this coating material may interfere with the measurement of target substances. Alternatively, the bounce of spherical atmospheric particles can be decreased with increasing relative humidity because these particles are hygroscopic and moist particles do not bounce significantly (Vasiliou et al., 1999). For our study, three different methods were tested for preventing the particle bounce: humidification of the incoming air and coating the impactor stages with two sticky materials, DS-515 (Dekati Ltd., Tampere, Finland) and ZEF-X10 (Zefon international, Inc., Ocala, FL). DS-515 is a spray-type of Apiezon-L grease, which is recommended by the ELPI manufacturer to minimize the particle bounce. Therefore, the ELPI data obtained using DS-515 coating served as a reference. However, DS-515 is not water soluble, which is a limiting factor for further analysis of particles collected on the impactor stages. In contrast, air humidification as well as coating plates with the water-soluble ZEF-X10 are compatible with microscopic analysis and could potentially be used with other analytical techniques based on aqueous sample processing, such as immunoassays.

DS-515 grease was sprayed evenly on aluminum foils of 25 mm in diameter (CF-300, Particle Instruments, LLC, MN) with a 22-mm stencil (DS-125, Particle Instruments) following manufacturer's instructions. The greased aluminum foils were placed on top of impactor stages of ELPI before the measurement started.

In our study, the humidification of fungal particles was achieved by mixing the released fungal particles with water vapor in a specially constructed humidification chamber. The chamber was placed between the FSSST and the ELPI. The final chamber design was selected from three differently designed humidification chambers after testing for their effectiveness in maximizing mixing while minimizing particle losses. The first two chambers were designed to introduce water vapor upstream against the incoming fungal aerosol. One of them injected water vapor straight toward the centerline of chamber, and the other injected vapor with an angle of 10° from the centerline making the air flow swirl inside the chamber to maximize aerosol mixing. However, this type of design was found to cause large amounts of particle losses by impacting moistened fungal particles on the interior wall of the chamber. The third type of humidification chamber, which was designed to introduce water vapor in parallel with the fungal aerosol flow, exhibited relatively small particle losses. Therefore, the third design was chosen as the experimental humidification chamber. This was, in turn, compared with the two sticky coatings (DS-515 and ZEF-X10) with respect to the ability to prevent particle bounce. The relative humidity inside the humidification chamber was continuously monitored with a moisture measurement system (Protimeter Plc, Marlow, England). Relative humidity was adjusted to 60–90% to avoid condensation on the chamber wall.

The water-soluble adhesive material, ZEF-X10, was specifically developed for impactor-type air samplers. ZEF-X10 was diluted with water, and the diluted solution was evenly spread on 25-mm aluminum foils. In order to get a thin coating layer with maximum viscosity, the ZEF-X10-coated aluminum foil was dried in an oven (Isotemp Premium Oven; Fisher Scientific Company, Pittsburgh, PA) for 1 h at 40 °C and then placed on the ELPI impactor stages for the measurement.

The three different bounce prevention methods were compared by measuring the concentrations of released fungal particles. One-month-old cultures of *A. versicolor* were utilized for this experiment. As described below, the ELPI data obtained with the ZEF-X10 coating and with the DS-515 coating were found to show comparable size distributions of fungal particles. Therefore, the former coating was used for further experiments.

2.4. Size and charge distributions of three fungal species

The concentration, size, as well as the charge distribution of the aerosolized fungal particles were measured three times for each fungal species. After the repeated measurements, fungal particles were eluted into 2 ml H_2O containing 0.02% Tween 80 by vortexing individual aluminum foil from each impactor stage for 2 min. Each fungal suspension was filtered onto a separate mixed cellulose ester (MCE) filter of 13 mm diameter and 1.2 μm pore size (Millipore Corp., Bedford, MA). The MCE filters were placed on glass slides and made transparent with acetone vapor for microscopic analysis as described by Adhikari et al. (2003). Spore counting for 12 slides obtained from the 12 impactor stages was conducted from randomly selected 40 microscopic fields using a bright light microscope (Leitz Laborlux S; Leica Mikroskopie und Systeme GmbH, Germany) to confirm the absence or presence of fungal spores on the impactor stages. The concentration of fungal fragments was determined from the ELPI data by combining particle concentrations obtained from all impactor stages where no fungal spores were observed. The upper size limit for the fragments was determined as the cut-off diameter of the highest impactor stage where no fungal spores were observed. For spores, the size range was determined as the cut-off diameters of impactor stages corresponding to the size of single spores of each species: *S. chartarum* (3.12–5.11 μm) and *A. versicolor* (1.99–3.12 μm). The spore concentration was determined from the particle concentration measured at those impactor stages by the ELPI. The concentration and the size of fungal spores and fragments were utilized as input data for fragment exposure for the respiratory modeling.

2.5. Respiratory deposition

The deposition of fungal spores and fragments in the human respiratory tract was calculated using a computer-based model, LUDEP 2.07 (ACJ & Associates, Inc., Richland, WA), with parameters representing a typical adult male and a 3-month-old infant. The calculations are based on the concentration data collected within each of the 12 aerodynamic size ranges measured by the ELPI when challenged with each of the two fungal species with distinctly different aerodynamic diameters of spores; *S. chartarum* (5 μm) and *A. versicolor* (2.5 μm). For each species, calculations were performed separately for two size fractions; one representing single spores and the other representing fragments. The total intake was expressed as the total number of inhaled fungal particles during the time of exposure, which was one hour in this simulation. The intake was calculated with the concentration of released fungal particles multiplied by the time-weighted mean breathing rate with breathing patterns and the exposure time (1 h). Breathing rates and time intervals that simulate normal indoor activities, but do not include typical outdoor activities such as work or sports activities were used for the modeling process. For an adult male, the breathing pattern included the following: $0.45\,m^3\,h^{-1}$ (sleeping; 55.0% of time), $0.54\,m^3\,h^{-1}$ (sitting; 15.0% of time), $1.5\,m^3\,h^{-1}$ (light exercise; 30.0% of time); and for an infant: $0.09\,m^3\,h^{-1}$ (sleeping; 71.0% of time), and $0.19\,m^3\,h^{-1}$ (light exercise; 29.0% of time) (ICRP, 1994).

The model calculates particle deposition into five regions in the respiratory tract: (1) anterior nasal region (ET1), (2) main extrathoracic region comprising the posterior nasal passages, larynx, pharynx, and mouth (ET2), (3) bronchial region (BB), (4) bronchiolar region (bb), and (5) alveolar–interstitial region (AI). The deposition efficiency of inhaled particles into the respiratory regions depends strongly on the particle aerodynamic diameter, d_a. It was calculated for two components: the aerodynamic deposition due to impaction and sedimentation, which is a function of d_a^2, and the thermodynamic deposition due to diffusion, which is an inverse function of d_a. Detailed formulas for calculating deposition in each of the regions are presented in ICRP (1994).

The output data were reported as % of deposition in total and regional respiratory tract separately for fungal fragments and spores. Likewise, the numbers of deposited particles in specific regions and overall in the respiratory tract were calculated for fragments and spores by multiplying the amount of intake by the % of deposition. The deposition ratio of fragments to spores was obtained by dividing the number of deposited fragments by that of spores.

3. Results and discussion

3.1. Aerodynamic characteristics of fungal particles

The size distributions of *A. versicolor* particles measured by the ELPI when using bounce-preventing methodologies are shown in Fig. 2. The concentration of released fungal particles, obtained for the 12 size classes utilizing the direct-reading capability of the ELPI, are plotted as a function of the aerodynamic diameter. With the traditional DS-515 coating, the size distribution showed a mode of the particle concentration at the aerodynamic diameter of 1.99–3.12 μm corresponding to the aerodynamic size of *A. versicolor* spores (Reponen et al., 1996). It was also observed that the concentration of particles smaller than 0.80 μm was comparable or higher than that of larger particles confirming our previous studies reporting the release of microbial particles smaller than spores from contaminated surfaces in large quantities (Górny et al., 2002, 2003). The results obtained with the water-soluble ZEF-X10 coating showed a similar size distribution as the DS-515 revealing a mode for fungal spores at the aerodynamic diameter of 3.12 μm. Humidification, however, resulted in distinctively different particle size distribution as compared with the two other methods used for the particle bounce prevention. No mode was observed in the fungal spore size range, and the concentration of particles with the aerodynamic diameter of 0.20 μm was close to zero as most of the time the ELPI measured negative value of current for that particular stage. When the humidification system was operated at lower relative humidity of 40–50% as compared to the normal

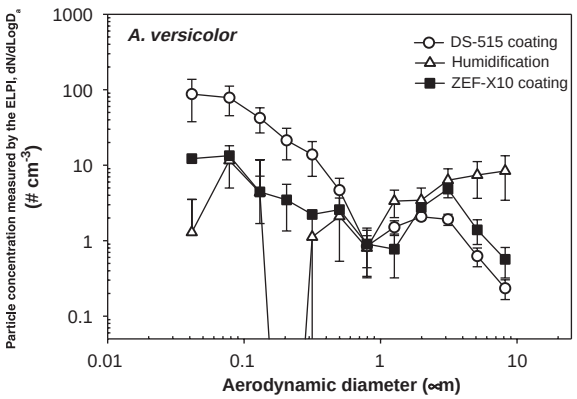

Fig. 2. The effect of the particle bounce preventing methods on the particle size distribution. The particle size distribution of aerosolized *A. versicolor* particles as measured by the ELPI with DS-515 coating on the impactor stages (○), humidification of the incoming air (△), and ZEF-X10 coating of the impactor stages (■). Each data point represents mean ± sd of three repeats. Arrows indicate corresponding *y*-axes for the particle concentration and induced current.

operation condition of 60–90%, higher particle concentrations were recorded at upper stages (0.50–8.18 μm), but more zero readings were obtained at small particle size ranges (0.13–0.32 μm). These zero readings appear to be caused by bounce-off of insufficiently humidified fungal particles. On the other hand, lower humidity reduced the loss of particles, which would otherwise have been deposited on the interior wall of the humidification chamber. In contrast to humidification, we did not observe negative current with either DS-515 or ZEF-X10 coatings when testing with *A. versicolor*. This suggests that these two coating media appear to prevent the bounce of *A. versicolor* spores about equally efficiently. As our further experiments required preparation of water suspensions from collected particles, the water-soluble ZEF-X10 coating was chosen for further experiments.

The fractional particle concentrations and the distribution of induced current measured with the ELPI for three different types of fungi are shown in Fig. 3. Microscopic counting of fungal spores collected on impactor stages was performed following the ELPI measurement and the results are presented in Fig. 4. For *S. chartarum*, the number of particles increased with decreasing size and no negative current was observed (Fig. 3a). The microscopic counting data, shown in Fig. 4a, demonstrated that no spores were found on impactor stage 7 and below (the corresponding aerodynamic diameters $\leqslant 0.79\,\mu m$) indicating that the ZEF-X10 coating effectively prevented the spore bounce. Microscopic examination confirmed that the large numbers of particles at lower impactor stages were truly fragments. The average concentration of released *S. chartarum* fragments (0.03–0.79 μm) obtained in our experiments was 380 particles cm^{-3}, which was about 514 times higher than that of spores in a size range of 3.12–5.11 μm. This result supports the evidence that *S. chartarum* spores can be difficult to aerosolize. For *A. versicolor*, the ELPI results showed that the spores and fragments were released in comparable amounts (Fig. 3b). The highest number of spores counted by microscopy was for stage 10 (3.12 μm) (Fig. 4b) coinciding with the mode measured by the ELPI. The microscopic examination confirmed that no observable spore bounce occurred onto impactor stages 6 and under.

In contrast, the ELPI measurement conducted with *P. melinii* showed different size and current distributions from those measured for *S. chartarum* and *A. versicolor* (Fig. 3c). High negative current was observed at impactor stage 5 within 30 s of the measurement resulting in zero concentration for the corresponding particle size (0.32 μm). Spores were observed on all impactor stages by microscopic counting demonstrating spore bounce-off in the ELPI (Fig. 4c). In order to confirm the bounce effect, the numbers of fungal spores, which saturate the surface of impactor stages theoretically, and the sampling time to collect those spores were calculated utilizing the particle concentration data measured by the ELPI. The single spore area on the impactor stage, which is occupied by a single spore of *P. melinii*, was calculated with the assumption that *P. melinii* spores have a spherical shape with the diameter of 3.0 μm. The total number of spores saturating the actual impaction surface with a single layer was obtained by dividing the actual impaction surface area

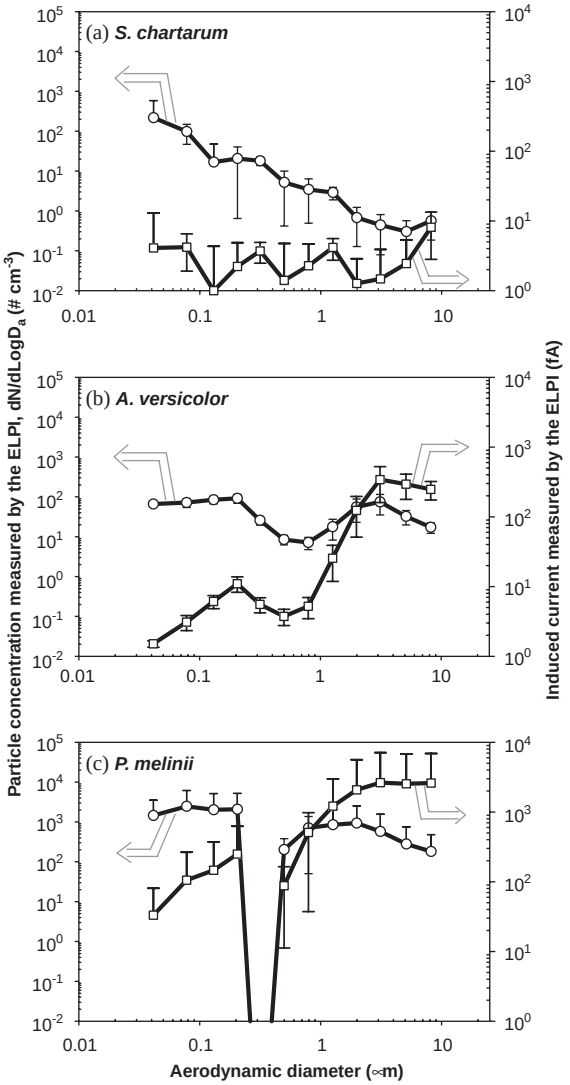

Fig. 3. The particle concentration (○; left *y*-axis) and induced current (□; right *y*-axis) measured by the ELPI for aerosolized particles of three fungal species: (a) *S. chartarum,* (b) *A. versicolor,* and (c) *P. melinii.* ZEF-X10 was used as the coating material on the ELPI impactor stages. Each data point represents the mean ± sd of three repeats. Arrows indicate corresponding *y*-axes for the particle concentration and spore count.

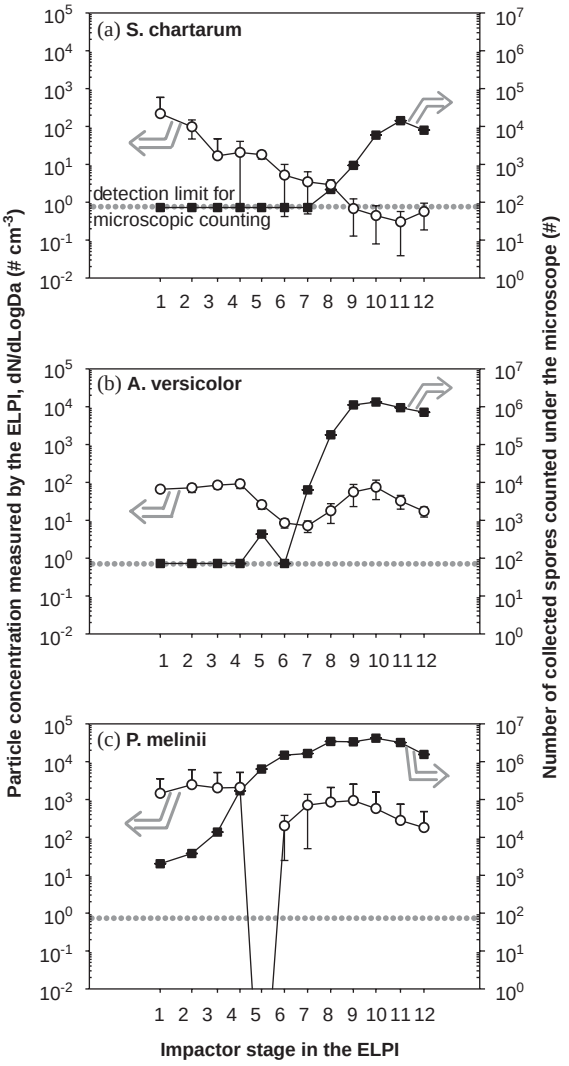

Fig. 4. The ELPI measurement vs. microscopic counting. The particle concentration measured by the ELPI (○; left *y*-axis) and the number of spores collected onto each ELPI impactor stage counted under a microscope (■; right *y*-axis) for aerosolized particles of three fungal species: (a) *S. chartarum*, (b) *A. versicolor*, and (c) *P. melinii*. The ELPI data are the same as in Fig. 3.

by the single spore area. The saturation time was calculated by dividing the number of saturating spores by the measured concentrations of particles in the size range of *P. melinii* spore (1.99–3.12 μm), and multiplying it by the inverse of the ELPI sampling flow rate (30 l min^{-1}). The saturation time was compared with the actual sampling time from the beginning of the aerosolization to the appearance of high negative value of current. It was found that the saturation times for stage 9 (1.99 μm) and 10 (3.12 μm) were 12 and 30 s, respectively, similar to the time when the negative current in the ELPI started appearing. Therefore, it was concluded that the negative current resulted from the particles that bounced from the saturated impactor stages. However, it is still not clear why the negative current and spore bounce did not happen for the two other fungal species, *S. chartarum* and *A. versicolor*. The spore bounce could be caused by the slightly higher concentration of *P. melinii* or by a higher aggregation rate of *P. melinii* aerosolized spores compared to two other species. *P. melinii* has a tendency to release spores in chains. These chains could break during impaction resulting in the escape of spores that have been separated during the break off (Trunov et al., 2001). Furthermore, the spiny surface of *P. melinii* spores could prevent a good contact between the spore and the collection surface (Trunov et al., 2001).

It was concluded that the particle distributions of *A. versicolor* and *S. chartarum* showed the real contribution of fragments, but that of *P. melinii* was masked by the spore bounce effect occurring on the upper stages of the ELPI. Based on the microscopic examination, the upper size limit for fragments was set at an aerodynamic diameter of 0.79 μm for *S. chartarum* and at 0.50 μm for *A. versicolor*. No clear fragment-spore border with respect to the particle size could be established for *P. melinii*, and, thus, this species was not considered for the respiratory deposition calculation, which was undertaken using the data on aerodynamic characteristics of the aerosolized fungal particles.

As mentioned earlier, a clear separation of fragments from spores for *S. chartarum* and *A. versicolor* was achieved successfully by the ELPI with the ZEF-X10 coating. The ZEF-X10 medium was found to be compatible with the microscopic analysis used for the quantification of fungal spores on the impactor stages. However, fungal fragments are too small to be quantified by microscopic analysis. Therefore, an attempt was made to test the immunological reactivity of fungal fragments as well as spores from samples collected on the ZEF-X10 medium. To test the compatibility of the ZEF-X10 medium with the immunoassay protocol, fungal suspensions with ZEF-X10 medium were prepared. Fungal particles of *S. chartarum* and *A. versicolor* scraped from the sporulating agar plates were separately mixed in ZEF-X10 solution (110 μl of ZEF-X10 and 2 ml of sterilized filtered water) imitating fungal particles suspended from the ZEF-X10-coated aluminum foil. Enzyme-linked immunosorbent assay (ELISA) was performed for the suspensions as described by Schmechel et al. (2003). It was found that fungal particles remained in suspension inside the ELISA wells even after overnight incubation, and the resulting optical densities of fungal samples were very low. It seems that the high viscosity of the ZEF-X10 medium blocked antigen adsorption to the ELISA well surface

resulting in correspondingly low ELISA values. This assay interference emphasizes the need to develop sample-processing techniques, which are compatible with sample analysis techniques to ensure the accuracy of the results.

3.2. Respiratory deposition of fungal particles

The efficiency of particle deposition in specific regions of the respiratory tract strongly depends on the particle's aerodynamic diameter and the breathing flow rate. Therefore, the difference between the size distributions of inhaled spores and fragments as well as the difference in breathing rates between an adult and an infant could result in different regional particle depositions. Model simulations were performed with two fungal species, *A. versicolor* and *S. chartarum*, which showed distinctively different concentration ratios of fragments to spores.

Fig. 5 presents the regional deposition fractions in percentages separately for inhaled fragments and spores representing 1 h of exposure. The total intake, which is the total number of inhaled fungal particles during the time of exposure, was calculated based on the concentration of fungal particles measured by the ELPI and modeled for age and activity-specific breathing pattern of an adult male and 3-month-old infant. For *S. chartarum*, which was found to release significantly greater number of fragments than spores, the total 1-h intake was about 500 times greater for fragments than that of spores [for an adult, 300×10^6 fragments h^{-1} and 0.6×10^6 spores h^{-1}; for an infant, 453×10^5 fragments h^{-1} and 0.9×10^5 spores h^{-1}]. For *A. versicolor*, the intake of fragments was three times higher than that of spores for an adult and an infant. The model calculations indicated that the total respiratory deposition fractions for fragments and spores were 27–46% and 84–95%, respectively. In general, 65–90% of inhaled spores for both fungal species were deposited in ET1 (anterior nasal region) and ET2 (main extrathoracic region), while only 3–15% and 2–5% of spores were deposited in AI (alveolar–interstitial region) and BB–bb (bronchial–bronchiolar region), respectively. More than half of the inhaled fungal fragments were exhaled again, and the exhalation was more efficient for an adult male than for a 3-month-old infant. Among fungal fragments retained in the respiratory system, 60% were deposited in AI, while only 14–15% of fragments were deposited in ET1 and ET2. Total and regional deposition fractions were slightly higher in an infant than in an adult for both fragments and spores. The total deposition of fragments for an adult and an infant were 27–41% and 33–46%, respectively, and those of spores were 84–92% and 93–95%, respectively.

Fig. 6 presents the fragment/spore deposition ratio, which is the number of fragments divided by the number of spores deposited in the respiratory tract. Generally, the deposition ratio is affected by (i) the intake ratio, which relates the number of fragments to spores inhaled into the respiratory tract, and (ii) the regional deposition ratio, which depends on the breathing rate and the aerodynamic characteristics of the inhaled particles including their size. As shown in Fig. 5, spores tend to be deposited in the upper airways and fragments in the lower airways, which were observed more prominently for a 3-month-old infant. For *S. chartarum*, the intake values resulted in a total deposition ratio of approximately 230 and 250 for an adult and an infant, respectively (Fig. 6a). This means that the number of deposited fragments of *S. chartarum* was 230–250 fold higher than that of spores. For *A. versicolor*, which showed comparable concentrations for fragments and spores, the total deposition ratio was less than 1 for both an adult and an infant (Fig. 6b). The regional deposition ratio was highest at the bronchiolar region (bb) and the alveolar region (AI) and lowest at the main extrathoracic region (ET2) for both age groups and for both fungal species. However, the scale of deposition considerably varied with age and fungal species. The regional deposition ratio of *S. chartarum* varied from 6150 (bb) and 4530 (AI) to 34 (ET2) for an infant, and from 1350 (bb) and 1340 (AI) to 36 (ET2) for an adult. For *A. versicolor*, the regional deposition ratio varied from 17 (bb) and 9 (AI) to 0.14 (ET2) for an infant, and 4 (bb) and 3 (AI) to 0.14 (ET2) for an adult. This shows that the deposition ratio in the lower airways but not the upper airways for a 3-month-old infant was 4–5 times higher than that of an adult male under the same exposure conditions. This suggests that the tendency of fragments to deposit into the lower airways is amplified 4–5 times for an infant when compared to an adult.

Once fine particles enter into the lower respiratory tract, they are difficult to clear. The clearance rate of fine particles from alveolar region to lymph nodes is reported as $0.00002\,d^{-1}$ compared to $1\,d^{-1}$ obtained for few micrometer size particles in the nasal region (ICRP, 1994). Experimental and epidemiological studies have reported that fine particles may increase inflammation and alter macrophage responses (Oberdörster et al., 1992; Renwick et al., 2004) as well as contribute to an increase in chronic obstructive pulmonary disease, pneumonia, and an overall decrease in lung function (Zanobetti et al., 2000; Peters et al., 1997). These studies have also suggested that the pulmonary toxicity of fine and ultrafine particles is due to their considerable total surface area. Normal lung development in children is particularly sensitive to pollutants until age 6–8 years (Burri, 1997; Cunningham et al., 1996) and our results suggest that exposure to fungal fragments may represent a greater relative risk than fungal spores during the early stages of childhood.

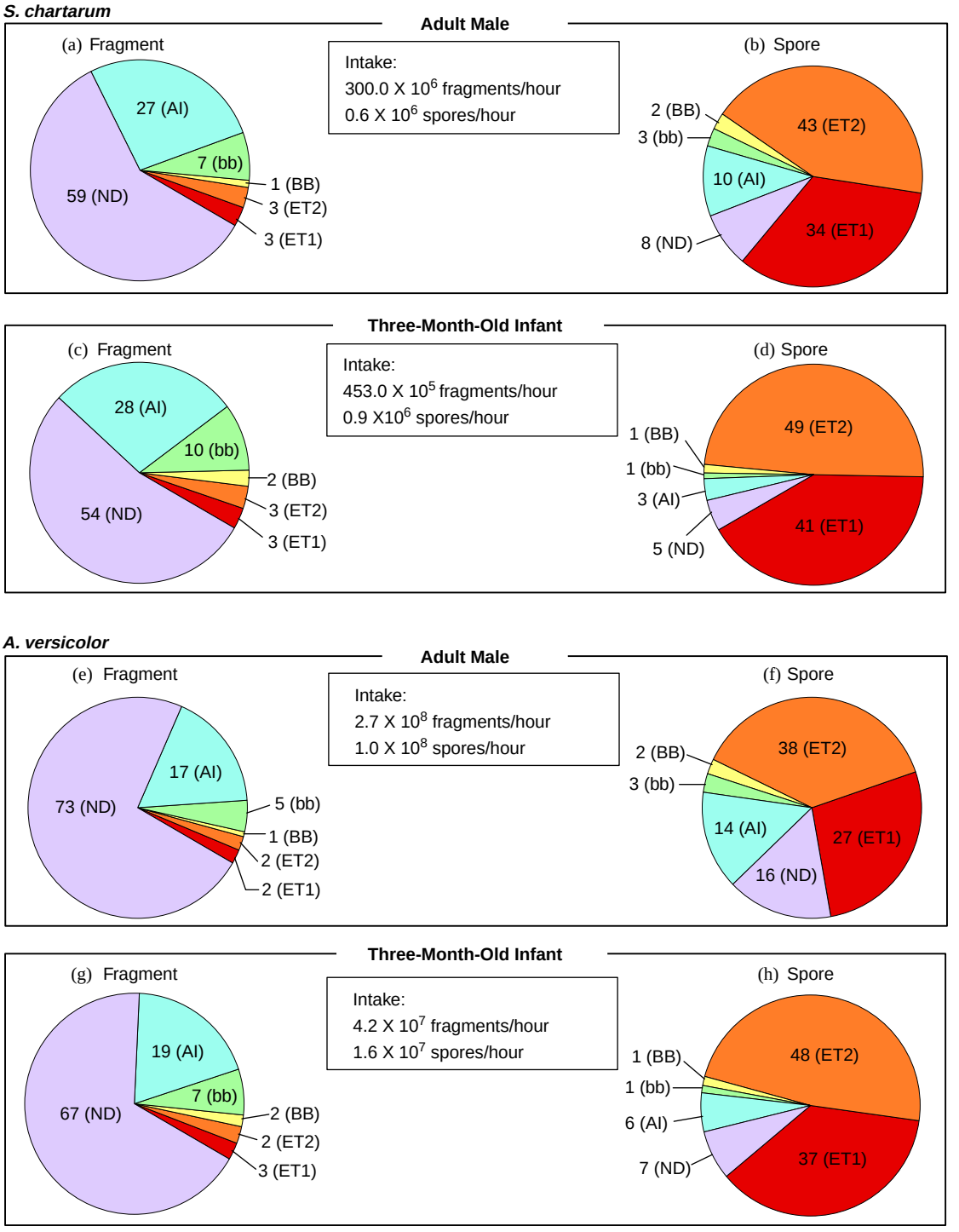

Fig. 5. Modeling the deposition of fragments and spores (%) into different regions of the respiratory tract for 1 h of exposure. ET1 indicates the anterior nasal region; ET2 indicates the main extrathoracic region; BB indicates the bronchial region; bb indicates the bronchiolar region; AI indicates the alveolar interstitial region; ND indicates not deposited particles. The total and fractional depositions were calculated for an adult male and a 3-month-old infant.

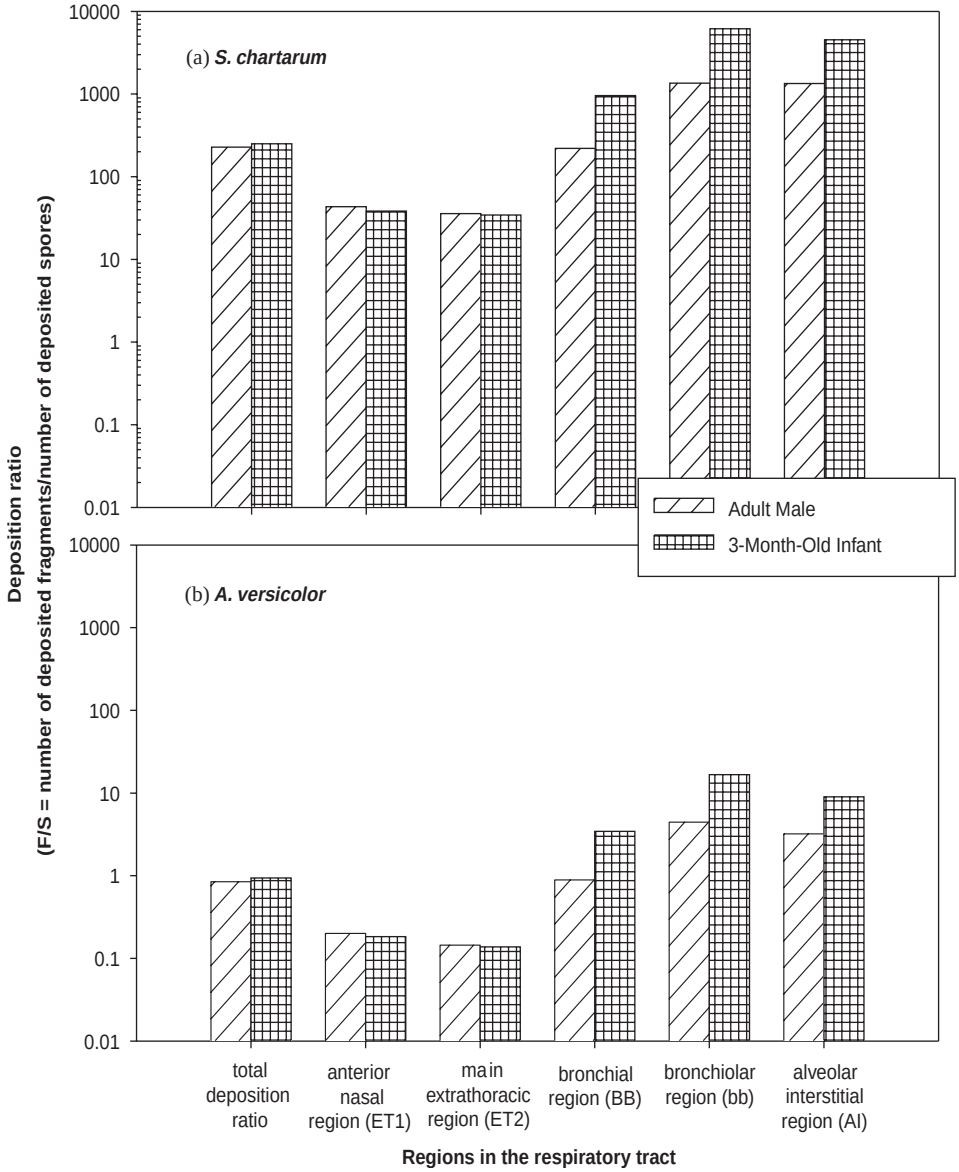

Fig. 6. The ratio of respiratory deposition of fragments to that of spores (F/S) for fungal particles of (a) *S. chartarum* and (b) *A. versicolor*.

4. Conclusions

In this study, the size distribution of fungal particles of aerodynamic diameters between 0.03 and 10 μm was measured with a direct-reading instrument, ELPI. The ELPI was found to discriminate fungal fragments and spores size-specifically when the collection surface of the impactor stages were coated with either of two adhesives: Apiezon-based DS-515 and water-soluble ZEF-X10. This study revealed that the fungal fragments released from contaminated surfaces outnumber spores and are more effectively deposited in the lower airways for a 3-month-old infant compared to an adult male. This trend was more significant for *S. chartarum* than for *A. versicolor*. Thus, the potential health effects associated with fine fragments of *S. chartarum* could be more pronounced in small children than in adults. While the mechanisms for adverse health effects associated with fungal exposure are not fully understood, recent toxicological characterizations have shown that fragments may contain mycotoxins and antigens.

In conclusion, the high number of released fungal fragments in combination with their potential to deliver harmful antigens and mycotoxins to the alveolar region

of the lung, especially for young children, suggest that future exposure measurements need to include fragment measurements. This would complement the current measurement strategies based on spore counts or sample cultivation and provide more realistic and comprehensive exposure profiles.

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