THE RELEASE OF FUNGAL SPORES FROM WATER DAMAGED BUILDING MATERIALS

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ABSTRACT

The release of spores from different typical indoor air fungi has been measured under controlled conditions. The fungi were cultivated for a period of 5-6 weeks on wallpapered wet sterilised gypsum boards at a relative humidity of approximately 97%. A special designed small chamber (P-FLEC) was placed on the gypsum board. The release of fungal spores was induced by an air-flow through rotating nozzles. The spores and other particles released from the surface were transported by the air flowing from the chamber through a top outlet to a particle counter and sizer. For two fungi (Penicillium chrysogenum and Trichoderma harzianum), the effect of changed humidity was also studied. For this experiment, the number of spores produced on the gypsum board and subsequently released was quantified. The method was found to give very reproducible results. It may also be applied for field studies and for generation of spores for exposure studies.

KEYWORDS: fungi, particulate matter, particle size distribution, surfaces, water damage

INTRODUCTION

Growth of micro fungi in water damaged buildings may be a cause of indoor air problems (1). Toxic and allergenic compounds from the fungi may be released as particles (e.g. spores), together with volatile organic compounds. The effect on the indoor environment caused by thegrowth of fungi will depend on this particle release. The release of spores and other fungal components from water damaged building materials is not sufficiently understood to allow risk assessment in buildings.

The purpose of this study has been to measure the release of fungal spores from growth on building materials. Based on a new method, the relationships between spore release and the type of fungal growth, and between spore release and changes in relative humidity have been studied.

METHODS

The experimental set-up illustrated in Figure 1 was applied in the study. The main component is the small chamber, P-FLEC. This instrument may be used for measuring the release or re-suspension of particles from a surface at an adjustable air-flow. The instrument was inspired by the Field and Laboratory Emission Cell (FLEC) (Chematec, Denmark) (2), which was developed for measuring the emission of gasses and vapours. In the P-FLEC (P for particle) emission of particles is induced by a well-controlled air-flow, directed at the surface in a 45°

angle through 10 rotating 0.8 mm nozzles in a tube. These 10 rotating nozzles were moved over a surface area of approximately 130 cm^2 for a period of one minute per rotation. The spores and other particles released from the surface were transported by the air flowing from the chamber through a top outlet to an Aerodynamic Particle Sizer (APS 3320, TSI Inc., USA) turned upside down. Particles were counted in 1 litre of air out of 10 liters directed at the surface. Particles released from the surface may alternatively be sampled on a filter. This small chamber was preferred from a windtunnel as it offers the possibility of field measurements in water damaged buildings.

The flow and the geometric lay-out of the P-FLEC were selected following a number of experiments with test particles on glass surfaces. The flow of 10 litres/minute through the 10 nozzles directed towards the surface in an angle of 45° gave by a rough estimate a mean velocity over the surface of 3 m/s. An air supply channel for the nozzle bar is built into the wall to avoid moving parts on the outside of the chamber. An extra channel above the nozzle bar is used for adding filtered air or removing excess air not drawn through the particle sampler.

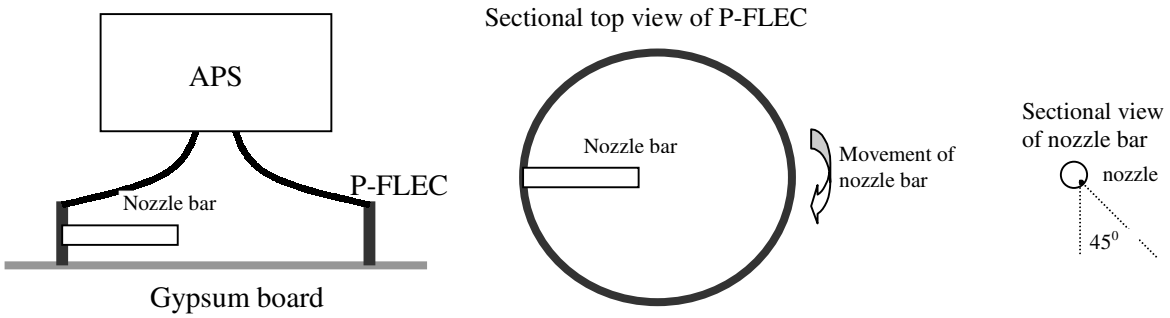

Figure 1 A schematic illustration of the experimental set-up. Air channels are not shown.

The measurements were performed on different fungi cultivated on pieces of sterilized wet wallpapered gypsum boards at a relative humidity of approximately 97%. Distilled milliQ water was utilized. Inoculation was performed by spraying 1 ml of a spore suspension (10^6 spores/cm^3) onto the piece of gypsum board using an atomiser. Following inoculation, the gypsum boards were cultivated in stainless steel boxes with tightly fitting glass covers. A saturated solution of potassium sulphate controlled the relative humidity at approximately 97%. The standard room temperature (20-22°C) was used. The fungi were incubated for 5-6 weeks before measurements were performed.

In the first experiment, the release of spores from nine different typical indoor air fungi was measured. These fungi were: *Acremonium sp.*, *Aspergillus versicolor*, *A. ustus*, *Chaetomium globosum*, *Cladosporium sphaerospermum*, *Penicillium chrysogenum, Trichoderma harzianum, Ulocladium sp.* and *Verticillium sp*. Each of these fungi was grown on three different 7×7 cm pieces of gypsum board. Three controls were not inoculated. After 6 weeks, the number of released spores was quantified by measurements using the P-FLEC and the APS.

In the second experiment, the two fungi *Penicillium chrysogenum* and *Trichoderma harzianum* were inoculated on larger (20×40 cm) pieces of wallpapered gypsum boards. These were cultivated in the same boxes as in the first experiment and under the same conditions for five weeks. In total, 24 gypsum boards were cultivated, 12 with each of the two

fungi concerned. After four weeks the relative humidity was reduced in some of the boxes by means of silica gel and the rest of the boxes were kept at the 97% level during the remaining incubation period. The purpose of this experiment was to study the effect of changed humidity. After 5 weeks two measurements were performed on each gypsum board, one at each end of the board. At one end, the released spores were counted and sized by the APS and, at the other end of the board, the spores were sampled on a 0.4 µm polycarbonate filter according to the CAMNEA method [3]. The number of spores was quantified by counting the spores in a microscope. Four samples from the fungi grown surface of each gypsum board were also taken. Three of these were used to count the number of spores by microscopy and the fourth was analyzed for the content of ergosterol [4].

RESULTS

Table 1 summarizes the results for the comparison of 9 different fungi in the first experiment. Figure 2 illustrates the size distribution of particles released from surfaces cultivated with *Penicillium chrysogenum, Trichoderma harzianum, Aspergillus versicolor* and *Ulocladium sp.* These size distributions have been measured by means of an Aerodynamic Particle Sizer (APS) and illustrate the number of particles as a function of aerodynamic diameter for the three repeated measurements for each fungi. The actual number of released particles has been a factor of 10 higher than shown in figure 2 as particles were only counted in 1 out of 10 liters of air blown through the P-FLEC.

The peak on the graph showing the size distribution for *P. chrysogenum* corresponds to the known spore size. Also the peak at ca 3 µm for *A. versicolor* corresponds to the known spore size, while the peak at ca 1µm may be other particle fragments released from the fungal growth. For *T. harzianum* it appeared that particles are released in agglomerates of 2 – 8 spores, probably due to a mucous layer covering the fungal growth. For *Ulocladium* sp. only very few particles were released.

Table 1. Measured number of released particles [Total no./measurement]

Fungus Meas. no.	**1**	**2**	**3**
Acremonium sp.	5	3	8
Aspergillus versicolor	$25 \cdot 10^3$	$29 \cdot 10^3$	$64 \cdot 10^3$
Aspergillus ustus	$1.1 \cdot 10^6$	$5.0 \cdot 10^5$	$1.2 \cdot 10^6$
Chaetomium globosum	$18 \cdot 10^3$	$71 \cdot 10^3$	$91 \cdot 10^3$
Cladosporium sphaerospermum	$4.6 \cdot 10^5$	$5.0 \cdot 10^5$	$4.8 \cdot 10^5$
Penicillium chrysogenum	$3.5 \cdot 10^6$	$2.8 \cdot 10^6$	$3.6 \cdot 10^6$
Trichoderma harzianum	$32 \cdot 10^3$	$28 \cdot 10^3$	$27 \cdot 10^3$
Ulocladium sp.	564	479	603
Verticillium sp.	147	107	158
Control	29	39	66

The number of spores released from *P. chrysogenum* was close to the saturation level for the APS, which means that coincidence errors with more than one particle in the measuring volume at the same time probably affects the result for *P. chrysogenum*.

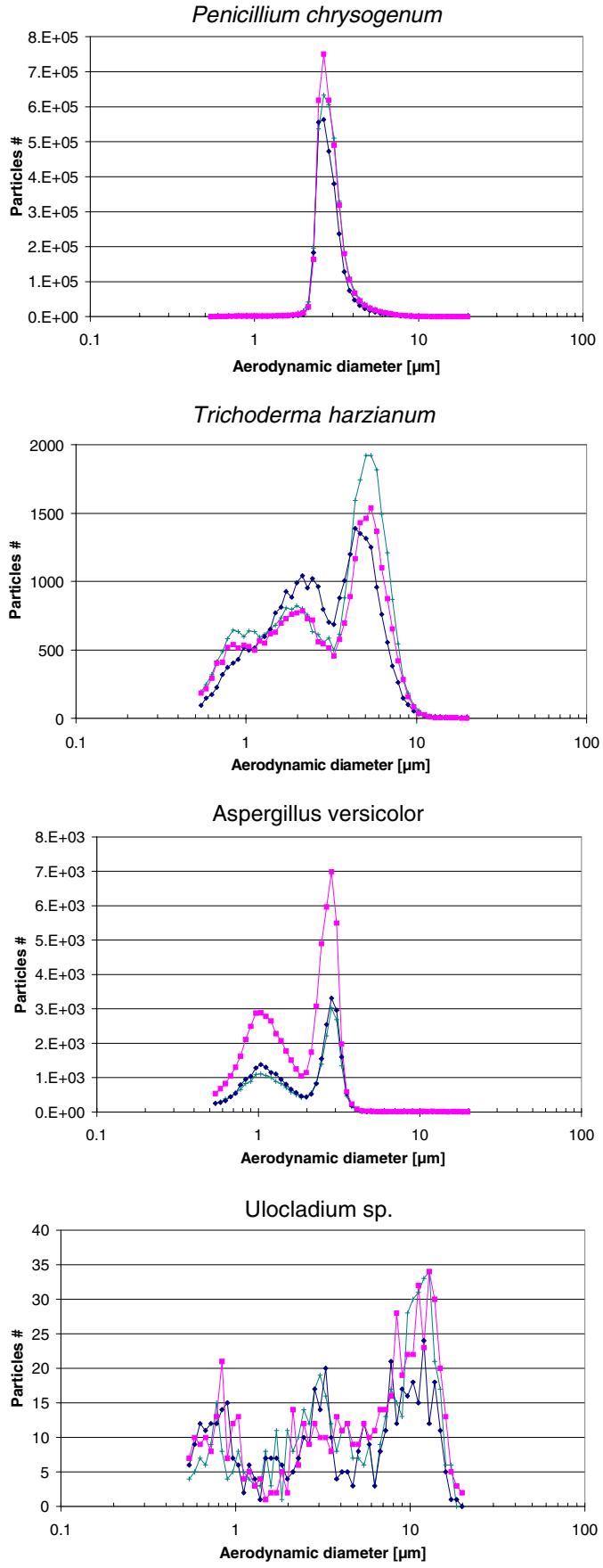

Figure 2. Size distributions for released particles

Table 2 summarizes the main results from the second experiment. A significant correlation was found for *T. harzianum* between spores counted on the filter and particles counted by the APS ($R^2 = 0.53$, $p = 0.017$). There also seemed to be a relationship between the relative humidity at the end of the cultivation period and the number of spores on the gypsum board ($R^2 = 0.30$, $p = 0.084$) for *T. harzianum*. A 2nd degree polynomial fit gave a regression coefficient of $R^2 = 0.58$.

Table 2. Mean values for the second experiment where the relative humidity was reduced after four weeks

Fungi	n	RH range %	Spores, board #/130cm^2	Spores, filter #/10 liter air	Particles, APS* #/1 liter air	Ergosterol µg/cm^2
T. harzianum	11	46 – 96	$1.62 \cdot 10^9$	$1.72 \cdot 10^6$	$2.56 \cdot 10^4$	12.7
P.chrysogenum	10	46 – 96	$9.08 \cdot 10^9$	$1.02 \cdot 10^8$	$2.69 \cdot 10^6$	3.4

* The number of released particles were a factor of 10 higher as the APS counted particles in 1 of 10 liters of air.

From Table 2, it appears that approximately 1 out of 1000 spores was released on average from gypsum boards with *T. harzianum* when looking at spores sampled on a filter. For *P. chrysogenum*, it seems that approximately 1 out of 100 spores was released on average.

DISCUSSION

In this study, very reproducible results were found when measuring the release of spores from different fungi. The experimental set-up was found to be very feasible for measuring the release of spores in the laboratory. The method may also be applied in the field when the sampling is performed on a filter instead of using the APS.

The comparison of the particle release from nine different fungi illustrates how much spore release in a given situation will depend on the type of fungi and their respective growth habits under the given conditions. The second experiment shows that the fraction of spores released from the surface is different for different fungi. Furthermore, a relationship with relative humidity was found.

The difference in Table 2 between the number of released spores sampled on a filter and counted by microscopy and the number of particles counted by the APS may have two different explanations. For *P. chrysogenum*, the APS was saturated to some extent due to the high number of particles. For *T. harzianum*, a major part of the spores seem to be released in agglomerates which are counted as one particle in the APS but will be counted as individual spores in the microscope. The fact that the two measurements were performed at each end of the gypsum boards, will also introduce some uncertainty.

The release of fungal spores has also been studied in relation to the indoor environment by e.g. Pasanen et al. [4] and Foarde et al. [5]. Both found a relationship of spore release to relative humidity and air velocity, respectively, and applied colony forming units (CFU) as the main measure. One advantage of the method developed in the present study is the possibility of performing field studies. Also the application of a particle sizing instrument offers the possibility of detecting other particles than spores and to study the agglomeration of spores. The finding of fragments smaller than the spores from both *A. versicolor* and *T. harzianum* is interesting as it could explain why CFU concentration in indoor air of mouldy buildings with health effects is not significantly higher than in not mouldy buildings [7,8].

In field studies, the described method may be used for measuring the potential release of spores from different mouldy surfaces. This would provide information on the way in which different water damaged materials in a building contribute to the airborne spore exposure. The released spores could be sampled on filters for later analysis or counted by small portable particle counters.

In addition to measuring the release of fungal spores from water damaged building materials in the laboratory or in the field, the P-FLEC method may also be used for other measurements, e.g. the release of asbestos fibres from surfaces, the resuspension of accumulated house dust or the release of particles from deteriorated surface materials. By choosing different air-flows, the P-FLEC has the potential to simulate release of particles caused by different activities. Work in this field is in progress.

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