

Short communication

Indoor viable mold spores – a comparison between two cities, Erfurt (eastern Germany) and Hamburg (western Germany)

Background: In eastern Germany, the prevalence of allergies is lower than in western Germany for both children and adults. Several reasons for this fact have been discussed, although it is still not completely understood. One purpose of the epidemiologic study “Indoor and genetic factors in asthma and allergy” (INGA) is to compare exposure to mold spores in two German cities. Therefore, 405 homes in Erfurt (east) and Hamburg (west) were visited twice by trained investigators between June 1995 and May 1997.

Methods: Samples of settled dust were taken by vacuuming from the carpet in the living room. Sieved house dust was diluted and plated on DG18 agar. The analyses were carried out in duplicate in the same laboratory.

Results: No significant difference could be shown for the total and for single genera (*Alternaria*, *Aspergillus*, *Cladosporium*, and *Penicillium*) in concentration of spores of viable fungi in settled house dust between Erfurt and Hamburg. Seasonal variation of the mold picture, with highest values in August, could be identified both indoors and outdoors.

Conclusions: Because outdoor concentration is the main influence on indoor concentration of mold spores from June to October, we recommend sampling from November to May to evaluate exposure to indoor mold spores.

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Indoor allergens from house-dust mites, pets, cockroaches, and molds contribute to the development of allergic diseases. Indoor pollutants such as NO₂ and volatile organic compounds (VOC) are not primarily allergenic, but they could act as adjuvants that stimulate allergic reactions. Indoor factors such as temperature, humidity, and soft furniture, as well as ventilation rate, washing, and vacuum cleaning, influence the concentration of allergens and pollutants. Some of these factors show a relationship with allergic symptoms (21, 22).

In eastern Germany, the prevalence of allergies is lower than in western Germany for both children and adults (11, 12). Several reasons for this fact are discussed, although it is still not completely understood (21).

The epidemiologic study “Indoor and genetic factors in asthma and allergy” (INGA) investigates the influence of indoor conditions on the development of allergies. The aim of this part of the study is to compare distributions of mold spore concentrations in dust samples from Erfurt (eastern Germany) and Hamburg (western Germany).

Material and methods

The homes included in this study were inhabited by adults aged 20–44 years who participated in the case-control study INGA. A total of 204 homes in Erfurt (eastern Germany) and 201 homes in Hamburg (western Germany) were visited by trained investigators between June 1995 and May 1997 (14). The visits were repeated after a period of 6 months.

In each house, a dust sample was taken in a highly standardized way (14) by vacuuming with the same type of vacuum cleaner (Firm Philips, Hamburg, Type Flüsterjet Vitall 371, 1000 W) equipped with a special attachment (the ALK allergen mouthpiece, ALK, Hørsholm, Denmark) to collect dust on a paper filter. Dust was obtained from the carpet in the living room. For each floor, an area of approximately 1 m² was sampled for 2 min. The dust samples were stored at room temperature and the analyses performed within the next 10 days.

A total of 30 mg of sieved house dust (500 µm) (2) was analyzed for identification and quantification of viable fungi. Dilutions of dust in 0.9% NaCl were plated

on DG18 (dichloran-18% glycerol agar) with 0.100 g/l chloramphenicol added to prevent bacterial growth. The plates were incubated at 25°C for 10 days (20). All analyses were done in duplicate. The numbers of colony-forming units (cfu) were counted by the naked eye and expressed as cfu/g dust. Colonies were identified to genus by high-power light microscopy (Carl Zeiss Jena, Ergaval).

The relationship between the mold spore concentrations in Erfurt and Hamburg was examined by bivariate linear regression analysis. The Wilcoxon rank-sum test, two-tailed, was used to determine the level of significance between the concentrations. Statistical significance was set at the conventional 5% level. SPSS (Superior Performing Software Systems 8.0) was used for statistical analysis.

Results

Distribution of total viable fungi in living rooms in Erfurt and Hamburg

The response between the first and the second visit was 94% at Erfurt and 85% in Hamburg, totaling 89%. Altogether, 767 dust samples were taken, 51.6% of them in Erfurt and 48.4% in Hamburg. There was no statistically significant correlation between the first and the second samples due to the powerful influence of the seasons. We therefore treated the samples as random.

There was no statistically significant difference between the concentrations of spores of viable fungi in settled house dust in living rooms in Erfurt and Hamburg. The concentrations of spores of the genera *Alternaria*, *Aspergillus*, *Cladosporium*, and *Penicillium* were also similar in both cities.

Comparison of the frequency and concentration of some genera of molds

Penicillium was the most frequently detected genus in these homes. It was found in more than 80% of the analyzed dust samples, and the average percentage of spores in these samples was 19%. The genera *Aspergillus* and *Cladosporium* were detected in more than 50% of the homes, and the average percentage of spores in these homes was about 9%. The detection of spores of *Alternaria* was very small. A higher percentage of a genus pointed to the existence of an indoor source of fungi.

Concentration of spores of total viable fungi throughout the seasons

As Figs. 1 and 2 show, there was a similar seasonal variation of monthly mean concentration of spores in house dust in homes and in outdoor air. The peak values of mean indoor and outdoor concentrations were reached in August. From November to May, the

concentrations were similarly low, but, from June to October, the indoor and outdoor concentrations were both higher. For that reason, it was better for evaluation to view the periods November–May and June–October as the seasons winter and summer, respectively.

Moreover, during the months November–May, there was no statistically significant difference in the incidence of spores of total viable fungi, *Alternaria*, *Aspergillus*, and *Penicillium* in house dust in living rooms between both cities. But the concentration of spores of *Cladosporium* during the months November–May was significantly higher in Hamburg than in Erfurt ($P < 0.0001$).

Proposal of a threshold limit value

Data on fungi in settled house dust are limited. At present, not enough is known to permit setting of threshold limit values for fungi in nonindustrial indoor environments. Threshold limit values can be used to evaluate the results of dust sampling. Since the results are highly dependent on the methods used, the activities during sampling, and the season, these factors should be taken into account. It should be stressed that these threshold limit values are based on the range of values obtained in homes, and not on a health-risk evaluation. The threshold limit values were calculated with the 95th percentile as the baseline from the results of the 766 samples, analogously to chemical indoor analyses (Table 1). Because the outdoor concentration is the main influence on indoor concentrations of mold spores from June to October, we used the concentrations from November to May only (472 samples) for calculation of threshold limit values. Whereas *Alternaria* and *Cladosporium* were affected by the season, *Aspergillus* and *Penicillium* were independent.

This special 95th percentile as the baseline of our results is not identical with the category “very high” (concentrations higher than 120 000 cfu/g dust) (2). If this limit is exceeded in the months November–May, an indoor source is to be expected. Because of the powerful influence of the outdoor concentration on the level of mold spores in house dust, it is not possible to evaluate an indoor burden of mold spores in house dust from June to October.

Discussion

The concentration of spores of the genus *Cladosporium* from November to May was the only significant difference between Erfurt and Hamburg. Seasonal variation of the mold picture, with the highest values in August, can be identified both indoors and outdoors. The seasonal pattern is more pronounced outdoors than indoors.

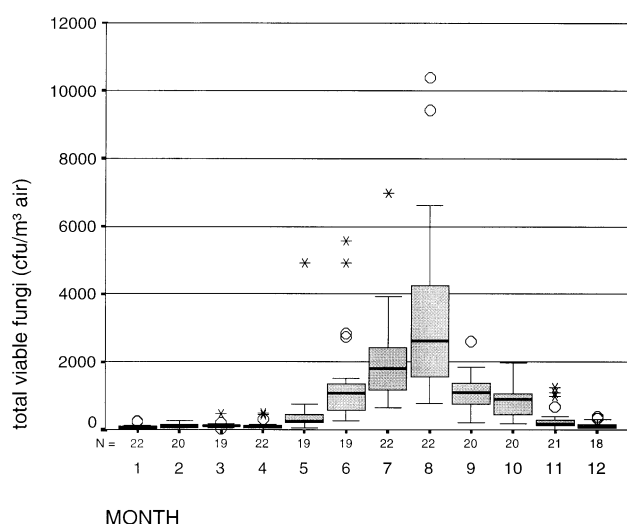


Figure 1. Comparison of concentrations of viable fungi in outdoor air in Erfurt by month.

There are different threshold limit values of indoor spores from genus to genus and for total viable fungi. Comparison of the percentage exceeding the threshold limit value for different seasons shows that season-specific thresholds are needed for several genera such as *Alternaria* and *Cladosporium*.

Because the results of dust sampling for viable fungi are highly dependent on the method used, the dust sampling was highly standardized, and identical methods were used for sampling at both locations. All analyses were done in duplicate in the same laboratory.

As stated before, both the qualitative and quantitative results of dust sampling for viable fungi are highly dependent on the method used, circumstances during sampling, the season, and the geographic area. For

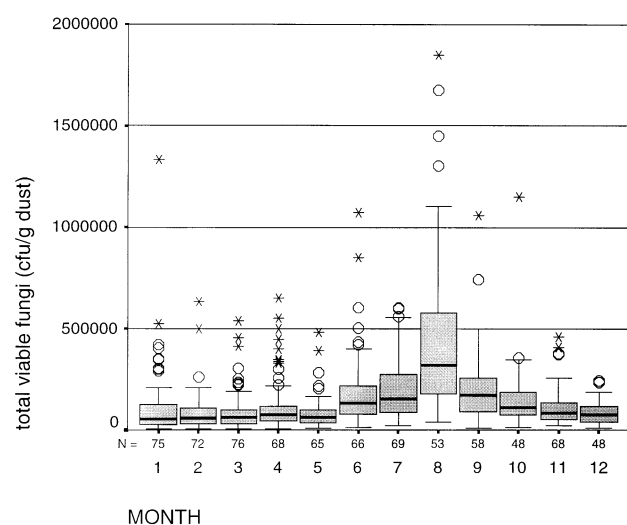


Figure 2. Comparison of concentrations of viable fungi in house dust by month.

Table 1. Threshold limit value from 95th percentile of 472 analyses of house dust from November to May (cfu/g dust) and of 766 analyses of house dust over whole year (cfu/g dust)

	95th percentile Nov.–May	95th percentile whole year
Total fungi	350 000	500 000
<i>Alternaria</i>	10 000	20 000
<i>Aspergillus</i>	50 000	50 000
<i>Cladosporium</i>	30 000	60 000
<i>Penicillium</i>	95 000	95 000

these reasons, reference values are difficult to establish. Recent studies on the presence of viable fungi in indoor house dust were carried out in Europe, Canada, and the USA. In urban areas throughout Canada during winter, Miller et al. (1988) (10) found a detection rate of 57% for *Alternaria*, 71% for *Aspergillus*, 67% for *Cladosporium*, and 80% for *Penicillium* on malt agar. The average value of 4.3×10^5 cfu/g dust was higher than the average value for our study, which was 1.6×10^5 cfu/g dust.

Analyses by Gravesen (5) of 100 dust samples in private homes in Denmark throughout the year showed similar results, but the dust was spread directly on V8-agar. The detection rate of *Aspergillus* was 48%. Studies in schools and offices (6) showed different frequencies for *Aspergillus*. In schools, the detection rate was 25% and in offices 55%. Wood et al. (25) collected dust in 84 private homes in the USA. The dust was also spread directly on V8-agar; the median was 2.4×10^3 cfu/g dust.

Recently, dichloran (18% glycerol agar [DG18]), developed by Hocking & Pitt (8), has proved to be useful in indoor biologic studies (18). It is intended to be a selective medium with low water activity for isolation of xerophilic fungi, but many of the common fungal species in indoor dust can also be isolated, although some hydrophilic fungi may not show up. Dichloran restricts the growth of the colonies, facilitating the counting and isolation of all species present. Through the use of DG18, the proof rate of *Aspergillus* could be increased from 2% to 10% and the detection rate from 32% to 60% during the first main phase in comparison with the pilot phase. Ostrowski et al. (13) also obtained higher cfu/g dust on DG18 than on malt extract agar. Because no single method or cultivation medium is sufficient to detect all the various indoor molds, a combination of air sampling, direct microscopic examination, and cultivation on both standard and low water activity media is recommended by Samson (15).

The presence of fungi shows wide seasonal differences (4, 24). For most genera, the highest numbers in the outdoor air are found during summer and autumn. During these seasons, the outdoor air is the main source of fungi in the indoor air. Recent studies indicated that the outdoor air spora influence the presence of fungi in

indoor environments, but the indoor air spora are not a simple reflection of the presence of fungi in outdoor air. Indoor sources of spores may also be present, and this is decisive for the comparison (3, 19). Hirsch & Sosman (7) showed that decreased occurrence of *Cladosporium* and *Alternaria* in winter was statistically significant ($P < 0.01$). *Aspergillus* occurred at approximately the same rate throughout the year. *Penicillium* showed only a small decrease in winter. Hirsch & Sosman (7), outdoors in Milwaukee (USA), obtained peaks of *Cladosporium* in June and July and peaks of *Alternaria* in September and October. Indoors in Germany, Schata et al. (16) obtained a peak of *Cladosporium* on carpets in living rooms in August. Senkpiel & Ohgke (17), outdoors in Lübeck (Germany), obtained a peak of total viable fungi in August just as in Erfurt.

The presence of fungi in house dust also seems to be an important source of airborne spores in the indoor environment. In house dust, species belonging to the following genera are commonly isolated: *Cladosporium*, *Penicillium*, *Alternaria*, *Aspergillus*, *Eurotium*, *Mucor*, and *Wallemia* (1, 2, 7, 9, 23). This ranking reflects the

relative occurrence in outdoor spore counts. The number of cfu/g dust ranges from $< 10^2$ to $> 10^6$.

Verhoeff et al. (20) compared analytic methods and their value as estimators of potential exposure of fungal propagules in house dust. The highest mean number of cfu/g dust was obtained by suspension of at least 100 mg dust in a peptone or sucrose solution in a ratio of 1:50 (w/w), followed by 10-fold dilution and plating on DG18 agar (geometric mean approximately 60 000 cfu/g dust). This method is comparable to ours in this study (geometric mean approximately 97 000 cfu/g dust). Because of the refined material and methods, the comparability with other authors, and the parallel sample period time over all seasons, the differences in concentrations of indoor viable fungi between Erfurt and Hamburg have a sound scientific basis.

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