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Normal Range Criteria for Indoor Air Bacteria and Fungal Spores in a Subarctic Climate

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Abstract

Indoor air bacteria and fungal spore levels were studied in 71 non-complaint homes. The data were analyzed according to the season and the higher limit of the range within which 95% of the cases fall was computed. On the basis of the data the following highest normal levels are proposed for winter: for bacteria 5000 cfu/m³ and for fungal spores 500 cfu/m³. The recommended levels apply in a subarctic climate for urban and suburban homes when the measurements are made using the same method as in this study. We recommend that if abnormal indoor sources are suspected, indoor samples should be taken in winter when the ground is frozen and covered with snow. At that time, the background levels are at their lowest and the abnormal indoor sources are most easily detected. The recommended levels should not be used as an indicator of a health risk, but as an indication of abnormal indoor sources or insufficient ventilation.

KEY WORDS:
Criteria, Fungi, Bacteria, Airborne, Indoor, Subarctic.

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Introduction

Bioaerosols form a large group of indoor air pollutants originating from different sources. They include bacteria, fungal spores, pollen, algae, protozoa and skin scales. In some cases, abnormal bioaerosol sources may develop in the building, causing excess exposure to the bioaerosols and health consequences among the occupants. Hence, some criteria are needed for hygienic evaluation of bioaerosol levels in indoor air. So far, no official criteria exist for bioaerosol levels; however, some recommendations have been presented.

In West-Germany, Ohgke et al. (1987) studied airborne fungal spores in 130 rooms of 11 office buildings with water damages. Based on measurements both in rooms with apparent fungal colonization and in rooms without visible mold growth, they suggested that indoor air concentrations above 100 cfu/m³ are indicative of fungal colonization of building materials. The measurements were made with a slit sampler on saboraud dextrose agar.

Holmberg (1987) from Sweden suggested that levels of *Aspergillus* spp. should be lower than 50 cfu/m³. The recommendation was based on measurements in 13 complaint homes with a May's impinger and V8 and malt extract agars. The study did not include any reference homes.

In Canada, Miller et al. (1988) suggested the following standards for fungal spores during winter:

- Some fungi, e.g. pathogens and toxigenic fungi are unacceptable in indoor air.
- If there is only one species present, concentrations of more than 50 cfu/m³ require further investigation.
- If there is a mixture of species other than pathogens and toxigenic species, concentrations less than 150 cfu/m³ are acceptable.
- If the species present are mainly *Cladosporium* or other common phylloplane fungi, concentrations up to 300 cfu/m³ are acceptable.

These recommendations were based on measurements in 50 Canadian homes, of which 70% had

been associated with health problems. Therefore, only about 16 normal homes were included in the material. The measurements were made using two methods. The first was a RCS centrifugal air sampler with rose bengal malt extract agar strips and the second was a filter sampling with rose bengal malt extract agar and malt extract agar with sucrose. The cut-off size of the RCS sampler has been reported to be about 3.5 μm (Macher and First, 1983), whereas the median diameter of indoor air bacteria and fungal spores has been reported to be about 3 μm (Macher et al., 1991). Thus, the collection efficiency of the RCS sampler for indoor air microbes is poor.

The American Conference of Governmental Industrial Hygienists (ACGIH) Committee on Bioaerosols suggested that a situation in a complaint environment can be considered unusual when the levels of the bioaerosols are at least an order of magnitude higher than those that commonly occur in control, symptom-free environments or if the organisms differ between the control and the complaint environment (ACGIH, 1989). In addition, the Committee recommended that, for saprophytic fungi, indoor air levels should be lower than those outdoors, and the taxa should be similar indoors and outdoors. The Committee also stated that the degree of the indoor/outdoor difference varies with ventilation mode: naturally ventilated interiors bear more resemblance to the outdoors than those with mechanical ventilation. Mechanically ventilated interiors, even those with minimal filtration, should have indoor fungus counts that are less than half of outdoor levels. However, the report did not include any data for the criteria presented. Malt extract agar for fungal spores and casein soy peptone agar and nutrient agar for bacteria as well as several different samplers were recommended.

Reynolds et al. (1990) from Minnesota, USA, put forward the view that airborne concentration of fungi exceeding 500 cfu/m^3 indicate an abnormal condition in the indoor environment. The suggestion was based on the measurements in six residential and office environments in response to health complaints. The study did not include any reference buildings, but comparative samples were taken outdoors and inside the buildings in the rooms where no complaints had been noted. The samples were taken with Andersen impactors using the stages 3 and 6 on inhibitory mold agar or sabouraud dextrose agar.

The above-mentioned criteria levels vary widely. This is partly due to different sampling methods. In none of the criteria has seasonal variation of the bioaerosol levels been taken into account. However, in a subarctic climate, such as in Finland, the seasonal variation, which is caused by the snow cover, is very distinct (Pasanen et al., 1990). Above all, the basis of the criteria varies; some have been based on measurements in complaint and some in non-complaint homes or offices. None of the previous reports have presented exact information on the grounds, either statistical or medical, on which the recommended levels have been based.

Evidently, not enough dose-response data are available to set any health-related criteria on bioaerosol levels. However, the general quality of indoor air is often described using bioaerosol measurements. These measurements can be used to detect mold problems in buildings. Almost no data are available regarding the range of bioaerosol levels in normal home environments where no microbial growth has been detected and no health problems due to indoor air quality have been suspected. We wanted to identify this normal range in order to be able to evaluate the levels measured in problem homes.

In the absence of any basis for general health criteria, we used a statistical approach in this study to determine the normal range of the bacteria and fungal spore levels in indoor air, and to propose hygienic criteria based on these data.

Materials and Methods

Indoor bacteria and fungal spore levels were measured in 71 relatively new urban and suburban homes with no identified construction damage or building-related health problems. The houses were typical of the Finnish urban and suburban building style. Of the homes, 48 were single family houses and 23 were apartment homes. Sixteen homes had a natural, 15 homes a mechanical exhaust and 40 homes a mechanical supply and exhaust system. The samples were collected in different seasons during 1983-1987.

The samples were taken with 6-stage cascade impactors (Model 10-800, Andersen Samplers, Inc., Atlanta, Ga) on tryptone-glucose-yeast agar for bacteria and rose bengal malt extract agar (Fries, 1943) for fungal spores.

The bacterial samples were incubated 5-7 days and the fungal spore samples 7-14 days at room temperature. The concentrations were counted as col-

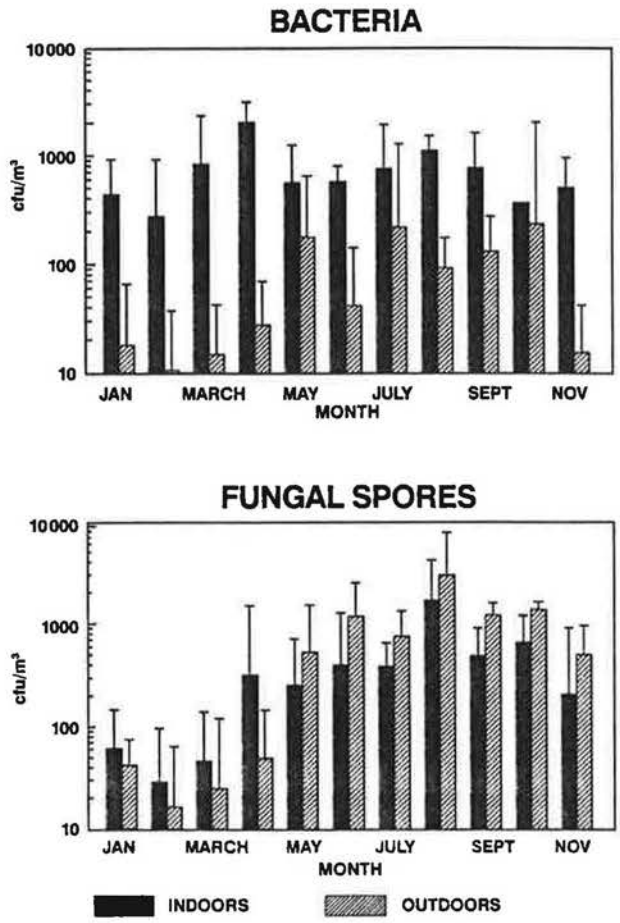

Figure 1. Monthly averages of bacteria and fungal spore concentration in indoor and outdoor air. The levels were higher in summer than in winter both indoors ($p < 0.01$ for bacteria and $p < 0.001$ for fungal spores) and outdoors ($p < 0.001$ both for bacteria and fungal spores).

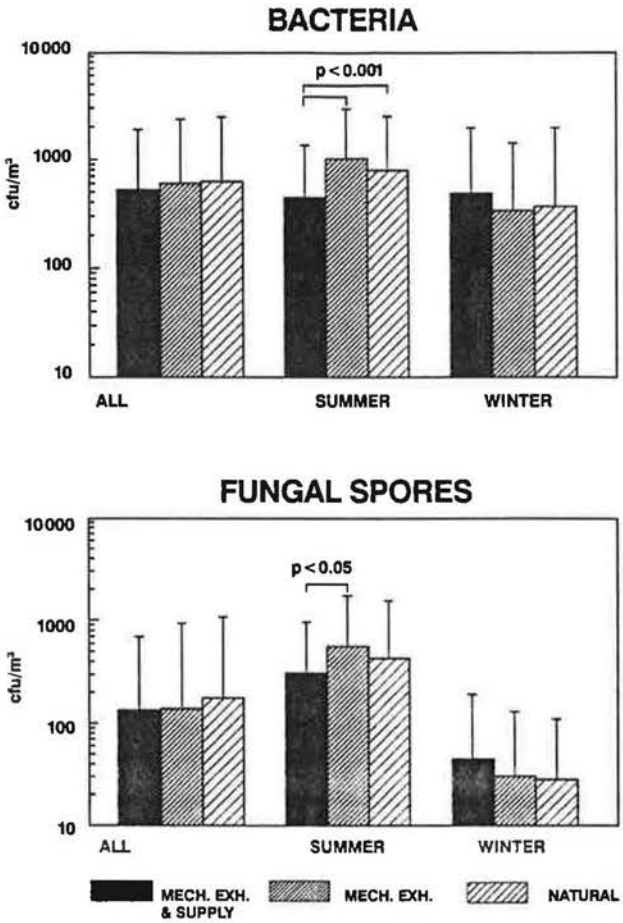

Figure 2. Indoor bacteria and fungal spore levels in homes with different ventilation systems. In the summer the levels were lowest in homes with a mechanical supply and exhaust ventilation system and highest in homes with a mechanical exhaust ventilation system ($p < 0.001$ for bacteria and $p < 0.05$ for fungal spores).

ony-forming units m^{-3} (cfu/m^3) using positive hole correction (Andersen, 1958).

The data were divided into two groups depending on the snow cover on the ground. The period from December to March was classified as winter and the period from May to October as summer. Because the ground was only partially covered with snow in November and in April, the data from these months were not included in the seasonally classified data.

Statistical tests were done with the SPSS-program package. The normality of the distributions was checked with the Kolmogorov-Smirnov test. Because the distributions of both the concentrations and the I/O ratios were lognormal, all statistical tests were done with log-transformed data. Seasonal variation was tested with t-test and the differences between the homes with different ventilation systems with one-way analysis of variance. The Scheffe test was used to locate the difference that one-way analysis of variance indicated.

Results

Seasonal Variation of the Levels

In the outdoor air, levels of both bacteria and fungal spores were an order of magnitude lower in the winter than in the summer (Fig. 1). This is natural because the soil and vegetation, which are undoubtedly the main sources for outdoor air bacteria and fungal spores, were frozen and covered by snow.

In indoor air, bacteria levels did not show as clear a seasonal variation as in the outdoor air. The geometric mean of indoor bacteria levels was higher in the summer than in the winter but the ranges were of the same order of magnitude during both seasons (Fig. 1, Table 1). In addition, the indoor levels were

Table 1 The geometric means (GM) and the higher limits of the ranges within which 67% (GM*GSD) and 95% (GM*GSD²) of the cases fall for bacteria and fungal spore levels. Each case (n) is the geometric mean of 1-3 samples, which were collected in one home during one day.

Sampling site and season	n	Bacteria			Fungal spores		
		GM	GM*GSD	GM*GSD²	GM	GM*GSD	GM*GSD²
					(cfu/m³)		
Outdoor air							
whole year	141	60	300	1600	230	2000	15000
summer	86	150	500	1500	950	2000	4600
winter	48	12	50	150	20	100	300
Indoor air							
whole year	162	570	1600	4500	150	700	3500
summer	86	690	1500	3400	410	1000	2400
winter	67	410	1400	4700	40	150	500

higher than the outdoor levels in 90% of the cases. The geometric mean of the I/O ratio (Indoor/Outdoor ratio) was 4.5 in the summer and 27.5 in the winter. This indicates that indoor sources dominate the indoor air bacteria flora during the whole year.

The levels of indoor fungal spores were an order of magnitude lower in the winter than in the summer, similar to the outdoor air levels. In addition, the fungal spore levels were lower indoors than outdoors (I/O < 1) in 86% of the cases; the geometric mean of the I/O ratio was 0.4 in the summer and 1.4 in the winter. Thus, fungal spores from outdoor sources dominate the indoor levels in the summer. The effect of indoor sources on airborne levels can be determined most easily in the winter, when the outdoor levels are low.

There were differences between the homes with different ventilation systems only in the summer. The indoor levels of both bacteria and fungal spores were lowest in homes with a mechanical supply and exhaust ventilation system and highest in homes with mechanical exhaust (Fig. 2). The corresponding outdoor samples showed no differences.

Proposal for the Upper Limits of the Normal Range

A much used statistical practice in defining a normal range in biomedical research is to use the ranges within which 67% or 95% of the cases fall. For the lognormal distribution these ranges are defined by

$$\exp(\ln GM +/- \ln GSD)\ (67\%) \quad (1)$$

$$\exp(\ln GM +/- 2*\ln GSD)\ (95\%) \quad (2)$$

where GM= geometric mean and
GSD= geometric standard deviation,

and

$$\ln GM = \frac{\sum \ln x_i}{N} \quad (3)$$

$$\ln GSD = \left[\frac{\sum (\ln x_i - \ln GM)^2}{N\text{-}1}\right]^{1/2} \quad (4)$$

where x_i = the concentration of fungal spores or bacteria in sample i, and
N = the count of cases.

Thus, the higher 67% limit is GM * GSD and the higher 95% limit is GM * GSD².

In our data the higher 67% and 95% limits in the winter are 1500 and 5000 cfu/m³ for bacteria and 150 and 500 cfu/m³ for fungal spores, respectively (Table 1).

Applying the above-mentioned 95% criteria for the normal range, we came up with the following proposal for an upper limit of the normal range in urban and suburban homes in a subarctic climate in the winter:

bacteria: 5000 cfu/m³
fungal spores: 500 cfu/m³.

Discussion

For bacteria, and especially for fungal spore levels, the seasonal variation is considerable in a climate with distinctly different seasons. We recommend that if abnormal indoor sources of microbes are suspected, indoor air samples should preferably be taken in the winter when the ground is frozen and covered with snow and the outdoor air levels are low. The background levels are then at their lowest and the abnormal indoor sources are most un-

mistakably detected. In the summer, the high and variable outdoor air levels may hide the effect of any but the strongest indoor sources.

Hence, the proposed levels, 5000 cfu/m^3 for bacteria and 500 cfu/m^3 for fungal spores, are applicable in the winter in the subarctic climate, when the soil is frozen and covered with snow (from December to March in Finland). The same criterion can be applied for all ventilation systems, because in the winter there are no differences between the homes with different ventilation systems. Where the proposed upper limits of the normal range are repeatedly exceeded, this incidates abnormal indoor microbe sources. Insufficient ventilation may be another reason for abnormally high bacteria levels (Nevalainen et al., 1991).

In the summer, when the outdoor air levels of fungal spores range from 10^2 to 10^4 cfu/m^3, the indoor air levels exceed 500 cfu/m^3 most of the time. For bacteria, the indoor sources are dominating, but in the summer the high outdoor levels increase the indoor air bacteria levels also.

In the summer, the situation is even more complicated because of the effect of the ventilation system. We found that in the summer, the levels of both indoor bacteria and fungal spores were lowest in homes with a mechanical supply and exhaust ventilation system and highest in homes with a mechanical exhaust ventilation system. This contradicts the guidelines of ACGIH (1989) that state that mechanically ventilated interiors, with or without filtration, have lower levels of microbes than naturally ventilated interiors.

The outdoor air microbe levels were similar between the homes with different ventilation systems. Therefore, the differences between the ventilation systems were not caused by the outdoor levels. The low indoor levels in homes with a mechanical supply and exhaust ventilation system can be explained by supply air filtration that decreases the outdoor-indoor transport of micro-organisms (Reponen et al., 1989). Neither the mechanical exhaust ventilation system nor the natural ventilation system includes supply air filtration, but in summer, the ventilation rate is higher in homes with a mechanical exhaust system than in homes with natural ventilation (Reponen et al., 1991). The higher ventilation rate without any filtration increases the outdoor-indoor transport of micro-organisms and is evidently the reason for the highest indoor microbe levels in homes with a mechanical exhaust ventilation system.

If indoor air microbe levels need to be measured in the summer, the levels and the composition of microbes have to be compared to those in outdoor air and to those in reference buildings with similar ventilation systems. Therefore, each investigator or laboratory carrying out indoor air measurements should provide an adequate reference material which includes results of normal environments in the climate and cultural conditions in question.

In most cases, indoor air fungi are identified to genus level and the composition of the genera in the problem environment is compared to the outdoor or reference composition. This is an elaborate task and can only be done by an experienced person. However, it has been stated that the identification of fungi to the genus level is not sufficient for identifying indoor sources (Fradkin et al., 1987). With the present methods, identification to the species level is expensive and requires considerable experience in environmental mycology. Therefore, species level analysis cannot be used routinely in evaluating indoor air problems related to microbial contamination.

The recommended levels do not apply to farmhouses, where microbial levels are higher than in urban homes due to transmission from animal shelters (Pasanen et al., 1989).

The recommended levels should on no account be used as a measure of absence or precence of a health risk, but as an indication of abnormal indoor sources or insufficient ventilation.

It should also be kept in mind that the recommended levels apply only to data based on the same sampling method as in this study. Because of the different sampling methods, the comparison of our recommendation to previous criteria is impossible.

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