Influence of Season and Sampling Methods on the Measured Exposure to Indoor Microorganisms and their Inflammatory Potential

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1 Introduction

High exposure to microorganisms may cause adverse health effects such as headache and respiratory symptoms. However, exposure levels are measured using various sampling methods, and typically without consideration of seasonal variation. Other than making it impossible to compare data between studies, these issues may lead to false impressions when relating data to health effects. As part of the ongoing CISBO study (Sigsgaard et al. 2011), our focus is to compare different sampling methods while assessing seasonal variation of microbial exposure levels in normal homes. Different types of air- and surface sampling described below has now been completed for three seasons: spring, summer and fall 2010.To date, quantification of fungi, bacteria and endotoxin has been achieved for most samples. Yet to come is species identification and quantification of other microbial components, e.g. enzymes and beta-glucans. Furthermore, the inflammatory potential of the collected samples will be assessed and evaluated with respect to the microbial components of the samples.

2 Materials/Methods

Five Danish homes in the Copenhagen area are chosen amongst acquaintances for investigation of the exposure to microorganisms throughout an entire year. Each home is sampled twice within one week of each season. **Air sampling:** Gesamtstaubprobenahme (GSP) samplers mounted with Polycarbonate or Teflon filters are used to collect inhalable dust from the air, 1.5 meters above floor level, in 4 rooms of the home, along with an outdoor reference placed upwind. An impinger (SKC BioSampler), placed in one room, is used to collect airborne dust into a sterile liquid solution. The sampling time is between 5 and 6 hours. **Surface sampling:** Dust is collected by vacuuming surfaces minimum 1 meter above floor level onto filters. Sedimented dust is collected continuously throughout the year by the dust fall collector (DFC) (Würtz et al. 2005) and the electrostatic dust fall collector (EDC) (Noss et al. 2008). These lie side by side app. 2 meters above floor level, allowing dust to sediment for one-month (EDCs) or two-month (DFCs) periods. The dust from the DFCs is collected by vacuuming onto Polycarbonate filters. **Biological analyses:** Dust and bioaerosols are extracted from filters or EDCs in a sterile liquid solution. The suspension is then plated onto dichloran glycerol agar and nutrient agar with actidione for quantification of cultivable fungi and bacteria, respectively. Endotoxin is quantified by the Limulus Amebocyte Lysate test. The total inflammatory potential will be quantified by a cell-assay based on the production of reactive oxygen species by granulocyte-like cells (Timm et al. 2006).

3 Results

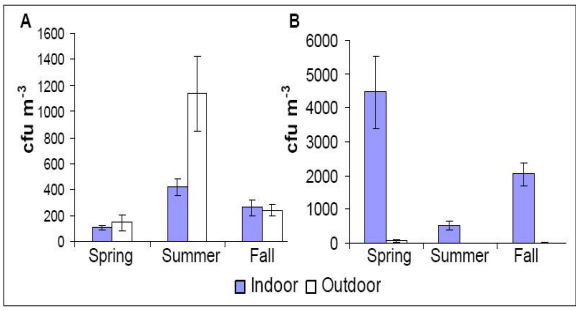

Figure 1: Mean concentrations of airborne cultivable fungi (A) and bacteria (B) (most outdoor bacteria levels were under the detection limit). Standard error (SE) is plotted.

Significant seasonal variation of indoor airborne fungi and bacteria, collected by GSP samplers, is seen between spring, summer and fall 2010 ($p = 0.0051$ for fungi, $p < 0.0001$ for bacteria,

N=24-37) (fig.1). Also, a strong correlation between indoor and outdoor fungi is observed ($r = 0.82$, $p < 0.0001$, $N = 24$).The GSPs show higher concentrations of both fungi and bacteria than the impinger, however the data from the two sampling methods still correlate (table 1).

Table 1: Mean concentrations of airborne cultivable fungi and bacteria sampled by the GSP or Impinger. Correlations (r) and p-values are calculated from log10 transformed data.

Conc. [cfu m^{-3}]	GSP	Impinger	N	r	p
Fungi	388	241	24	0.60	0.001
Bacteria	2231	1326	24	0.45	0.028

For surface dust sampling, the vacuum samples and EDCs strongly correlate regarding fungi, and show a seasonal pattern comparable to the air samples (fig 2). On the contrary, concentrations of fungi in the DFCs versus EDCs or vacuum samples do not correlate, nor do they follow the same seasonal pattern.

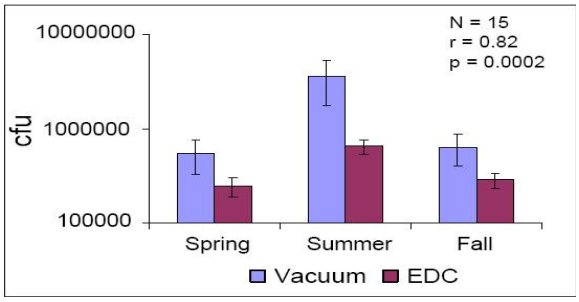

Figure 2: Mean concentrations of fungi in vacuum samples, presented as cfu g^{-1}, and EDCs, presented as cfu m^{-2}. SE is plotted.

The EDCs collect app. 8 times more fungi and 10 times more endotoxin than the DFCs (fig.3).

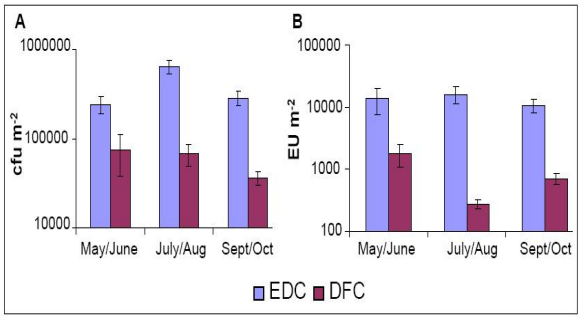

Figure 3: Mean concentrations of fungi (A) and endotoxin (B) in the EDCs and DFCs from all 5 homes (N=5). SE is plotted.

Further comparisons between the EDCs and DFCs also show more diversity in fungal groups in the EDCs (data not shown).

4 Discussion

Concentrations of airborne fungi increase from spring to summer, and decrease again in fall, whereas the opposite pattern is observed for bacteria. To our knowledge, this study is the first to assess seasonal variation of indoor fungi concentrations in Scandinavia, and indoor bacteria concentrations at all. The strong correlation between indoor and outdoor fungi may imply that the measured indoor fungi has an outdoor source. As for the comparison of methods, the impinger seems to underestimate exposure of both fungi and bacteria. Surprisingly, much higher levels of both fungi and endotoxin are observed in the EDCs compared to the DFCs. This may be due to the EDC's possible capability of keeping hold of the dust despite air currents within the home, whereas the flat smooth surface of the DFCs may not have this capability to the same extent. Alternatively, the electrostatic forces of the EDC may attract bacteria and spores to its surface. Nonetheless, the results from this study stresses the impact different seasons and sampling methods may have on measured microbial exposure levels and thereby sheds light on the difficulty of relating exposure to health effects.

5 References

Noss I, Wouters IM, Visser M, Heederik DJ, Thorne PS, Brunekreef B and Gert Doekes. 2008. Evaluation of a low-cost electrostatic dust fall collector for indoor air endotoxin exposure assessment. *Appl Environ Microbiol* Sep;74(18):5621-7.

Sigsgaard T, Clausen G, Gunnarsen L, Loft S, Riddervold IS, Wolkoff P. The Danish Centre for Indoor Air and Health in Dwellings – CISBO. Proceedings of Indoor Air 2011, paper nr. 421

Timm M, Hansen EW, Moesby L, Christensen JD. 2006. Utilization of the human cell line HL-60 for chemiluminescence based detection of microorganisms and related substances. *Eur J Pharm Sci* Feb;27(2-3):252-8.

Würtz H, Sigsgaard T, Valbjorn O, Doekes G, Meyer HW. 2005. The dustfall collector--a simple passive tool for long-term collection of airborne dust: a project under the Danish Mould in Buildings program (DAMIB). *Indoor Air* 15 Suppl 9:33-40.