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Airborne culturable fungi in naturally ventilated primary school environments in a subtropical climate

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HIGHLIGHTS

- 25 naturally ventilated school settings in a subtropical climate were investigated.
- Indoor culturable fungi were mainly driven by outdoor concentration.
- The concentration of culturable fungi is generally ≤1450 cfu/m^3 and I/O-ratio ≤2.
- Elevated levels indicate the presence of abnormal microbe sources indoors.
- The study guide future determination of criteria for assessing culturable fungi in a subtropical area.

GRAPHICAL ABSTRACT

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ABSTRACT

There is currently a lack of reference values for indoor air fungal concentrations to allow for the interpretation of measurement results in subtropical school settings. Analysis of the results of this work established that, in the majority of properly maintained subtropical school buildings, without any major affecting events such as floods or visible mould or moisture contamination, indoor culturable fungi levels were driven by outdoor concentration. The results also allowed us to benchmark the "baseline range" concentrations for total culturable fungi, *Penicillium* spp., *Cladosporium* spp. and *Aspergillus* spp. in such school settings. The measured concentration of total culturable fungi and three individual fungal genera were estimated using Bayesian hierarchical modelling. Pooling of these estimates provided a predictive distribution for concentrations at an unobserved school. The results indicated that "baseline" indoor concentration levels for indoor total fungi, *Penicillium* spp., *Cladosporium* spp. and *Aspergillus* spp. in such school settings were generally ≤1450, ≤680, ≤480 and ≤90 cfu/m^3, respectively, and elevated levels would indicate mould damage in building structures. The indoor/outdoor ratio for most classrooms had 95% credible intervals containing 1, indicating that fungi concentrations are generally the same indoors and outdoors at each school. Bayesian fixed effects regression modelling showed that increasing both temperature and humidity resulted in higher levels of fungi concentration.

1. Introduction

The association between moisture damage in school buildings, microbial growth due to excess moisture and the adverse health

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outcomes of the occupants, such as respiratory illnesses and allergy, has been reported in many studies (Aydogdu et al., 2005; Hussin et al., 2011; Meklin et al., 2002). However, there are no health-based guideline values for indoor dampness or microbes (Karvala, 2012; WHO, 2009). Measured microbiologic agents have shown less consistent association with health effects than qualitative assessment like visible dampness or mould odour (Karvala, 2012). In buildings with moisture and mould damage, indoor sources of fungi can be significant, and the overall mycobiota indoors may be extensive (Gutarowska and Piotrowska, 2007; Meklin et al., 2003).

Generally, there are no uniformly accepted, or validated, quantitative environmental sampling methods to assess exposure to mould and other agents associated with damp indoor environments (ACGIH, 2009; Frankel et al., 2012a). Andersen Impactor and Biotest RCS High Flow air samplers are widely used to detect and quantify bioaerosols, identify bioaerosol release from sources, assessment of human exposure to biological agents, and monitor the effectiveness of control measures (Li, 2011; Saldanha and Manno, 2008). It should be noted that although cultivation methods are convenient, being able to identify major fungal species with simple equipment and analysis techniques, they are slow and always selective and therefore underestimate the total fungal counts (ACGIH, 1999) and may ignore some clinically relevant moulds (Baxi et al., 2013; Holme et al., 2010).

Although, microbial levels by themselves should not be used as an indicator of a health risk, reference values for viable culturable fungi concentrations are needed in order to identify abnormal sources of microbes in different indoor environments in different climate regions.

Generally, the majority of the indoor airborne fungal population is derived from outdoor sources and is transferred inside through windows and doors (Burge et al., 2000; Levetin, 1995; Shelton et al., 2002). Fungal populations depend significantly on outdoor climatic conditions and meteorological factors, such as temperature and humidity (Bartlett et al., 2004; Frankel et al., 2012b; Wu et al., 2007). Nevertheless, when suitable conditions are present indoors, fungi may also grow on indoor building structures (Górny, 2004; Meklin et al., 2002). In such buildings, moisture and mould problems may manifest in elevated levels and/or altered types of culturable fungi in dust and air (Meklin et al., 2002; WHO, 2009). In addition, several other factors, such as the age of the building and presence of occupants and pets (Bartlett et al., 2004; Lehtonen et al., 1993) may cause variations in indoor fungal levels and explain differences between studies.

Indoor/outdoor (I/O) ratios are a direct numerical comparison of indoor fungal levels with outdoor levels, and can be used to determine if indoor spaces are contaminated with airborne microorganisms (Kim and Kim, 2007). Although it is generally accepted in the literature that, in non-damaged buildings, the microbiological concentration in indoor air is similar to outdoor air (I/O ratio is close to 1) (Bartlett et al., 2004), there are very few data available about I/O ratios in naturally ventilated non-moisture-damaged school buildings.

For properly maintained school structures and classrooms using natural ventilation in subtropical urban environments, this study aimed to (a) test the study's hypothesis that, under normal conditions, and without any major affecting events (e.g. floods) or visible mould or moisture contamination, the indoor culturable fungi concentrations is mostly driven by outdoor concentrations; (b) benchmark "baseline range" concentrations (and I/O ratios) of total culturable fungi, *Penicillium* spp., *Cladosporium* spp. and *Aspergillus* spp.; and (c) investigate the prevalence of fungal species and the effect of temperature and relative humidity on fungal levels.

It should be noted that the choice of the term "baseline" was not a straightforward one, however it was deemed the most appropriate, as it had to represent a situation when only the "normal" or "typical" sources were present, without any unusually high contributing sources. Such sources therefore contribute to the "baseline" situation.

2. Materials and methods

2.1. Study design, location and classroom characteristics

This cross-sectional study was carried out in 25 randomly selected primary schools (S01–S25) in the Brisbane Metropolitan Area, Australia. The participating school classrooms were naturally ventilated, using open windows. Some of the classrooms were equipped with ceiling fans, which were operated occasionally to improve thermal comfort. The selection criteria for schools, and detailed information about the classrooms are described in our other paper (Salonen et al., 2013) and are also available in the Supplementary information (SI). This study was conducted during teaching periods at S18–S21 in autumn (March–May), S08–S12 and S22–S25 in winter (June–August), S01–S03 in spring (September–November) and S04 in summer (December–February), regardless of the weather condition (e.g. rainy days).

Prior to sampling, a "walk-through" assessment was carried out to determine indoor and outdoor sampling locations. Room characteristics, with regards to moisture damage, cleanliness, floor type, and other possible bioaerosol sources, were also assessed in each studied classroom visually as well as via a questionnaire and information form (Appendix S1 in the SI). The classrooms had carpeted floors and there were no animals or pot plants inside the classrooms. Our inspections and interviews with school maintenance and management personnel showed that there was no visible moisture or mould in building structures at the time of the measurements, nor was there a history of moisture or mould problems (except at schools S09 and S10). Schools S09 and S10 were affected by the Brisbane flood in 2011, six months before the measurements were conducted. However, major clean-up and renovation works were conducted in the affected building structures and classrooms immediately after the flood waters receded. All of the wet material in these two affected schools were completely removed and replaced with new material and furniture. In other schools, no renovations were conducted during last two years. The classrooms were located in the ground level or one level above the ground.

The daily cleaning schedule during the measurement period included carpet vacuum cleaning and desk wiping in each classroom. Vacuum cleaning was conducted before or after school hours and desk wiping was often done once a week. Children were undertaking their normal classroom activities (reading and writing) during the sampling. Windows were mainly open (42 out of the 50 classrooms) during the measurements (generally the case during school hours in classrooms in) and there was no rainy (except mild rain in one school) or windy weather during sampling.

2.2. Sampling and instrumentations

2.2.1. Culturable fungi

Culturable fungi were collected at two indoor locations (classrooms A and B) and one central outdoor location (location C) within each school setting. All measurements were conducted during regular school hours and normal room activities (reading and writing) to reflect the conditions to which students and staff are exposed between the hours of 9 am and 3 pm. Measurements were taken at a height of 1.0 m above floor level, which is representative