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## **INFLUENCE OF OCCUPANCY AND BUILDING CHARACTERISTICS ON THE SOURCE STRENGTHS OF BACTERIA AND FUNGI IN THE CLASSROOM AIR OF PRIMARY SCHOOLS**

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### **INTRODUCTION**

Through resuspension and direct shedding, human occupancy significantly influences the airborne concentration and microbial composition of indoor air (Fox et al., 2005; Hospodsky et al., 2012; Noble et al., 1976; Qian et al., 2008; Qian et al., 2012). Improving microbial exposure models that account for occupancy requires (a) an accounting of size-resolved bacterial and fungal concentrations that are determined independently of the well known biases of culture methods (Amann et al., 1995), and (b) broader knowledge of bacterial and fungal emission rates associated with human occupants. This paper presents baseline characterization data for airborne concentrations and indoor emission rates of bacteria and fungi sampled in school classrooms in four different countries. The indoor environments are seven preschool or primary school classrooms, located in the USA (Salinas, CA and New Haven, CT Barnard School), Germany (Berlin), Denmark (Copenhagen and Arhus), and China (Happy and Mosque Daycares, Lanzhou).

### **METHODS**

At each school, samples were collected from indoor air and outdoor air during separate occupied and vacant periods. In addition to size-resolved particulate matter mass (non-viable cascade impactor) and number concentration (optical particle counter) measurements, quantitative polymerase chain reaction (qPCR) targeting universal fungal 18S rDNA and bacterial 16S rDNA sequences, was used to determine size-resolved concentrations of total airborne fungi and total airborne bacteria both indoors and outdoors. Using metabolically generated CO<sub>2</sub> to estimate air-exchange rates, we then applied a mass-balance approach to estimate size-resolved indoor emission rates from the concentration data.

### **RESULTS**

All measured indoor air concentrations were elevated during occupancy. Mean indoor to outdoor (I/O) ratios for occupied conditions were 70 times, 11 times, and 10 times greater than I/O ratios during unoccupied conditions for bacterial genome copy number (GCN), fungi GCN, and total suspended particle mass (PM), respectively, averaged across all size ranges and sampling sites. Median geometric mean diameters (GSDs) for the seven indoor occupied

settings were 6.5  $\mu\text{m}$  (1.8) for bacteria, 6.1  $\mu\text{m}$  (1.7) fungi, and 6.5  $\mu\text{m}$  (1.7) particulate matter mass. The measurement of size-distributed concentrations of both microbes and particle mass allows for exploring the relationships between biological aerosols and total aerosols. Overall, median mass fractions of bacteria ranged from 2 ppm to 150 ppm and fungal mass fractions ranged from 0.6 ppm to 200 ppm depending on the size bin and taking into account data for occupied conditions from all seven settings. The changes in this fraction with aerodynamic diameter follow the profile commonly observed with bacterial and fungal concentrations, with bioaerosol to total PM minima in the 0.4 to 1.1  $\mu\text{m}$  and maxima in the 4.7-9  $\mu\text{m}$  stages.

Average emission rates for bacteria, fungi, and PM were 10.7 GCN/h per person, 7.1 GCN/h per person, and 19.2 mg/h per person, respectively (GCN = genome copy number) and were variable among the different locations, ranging over one order of magnitude. Peak emissions occur at the same size (4.7 to 9 micron size bin) for bacteria, fungi, and particulate matter (Figure 1) and this emission size distribution closely follows the indoor occupied concentrations. Additionally, genome number-based emission rates for bacteria and fungi were transformed into mass emission rates and compared with particulate matter mass emissions. The rank order in mass emission rates was total mass>>fungi>bacteria.

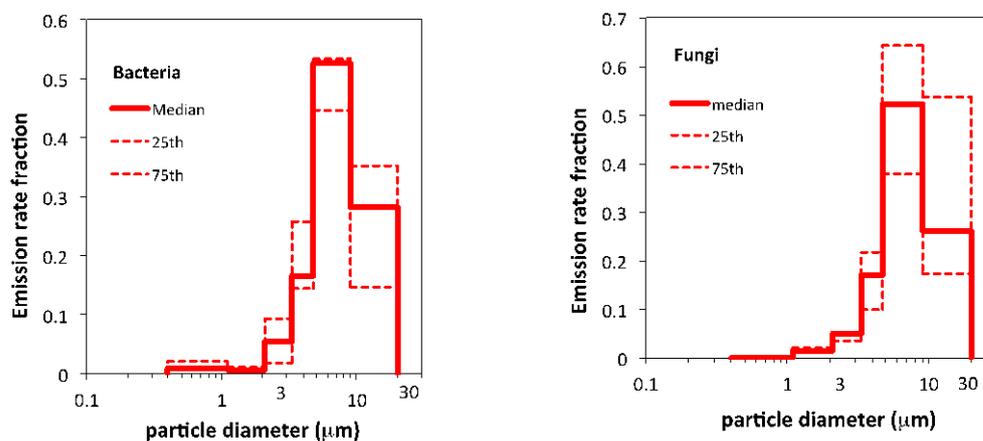


Figure 1. Normalized size distributions of bacterial (left) and fungal (right) emissions associated with occupancy. The emission rate fraction is defined as the emission rate in a particular size range divided by the total emission rate for all sizes. Plots represent 25<sup>th</sup> and 75<sup>th</sup> percentile values, separate by a median for the seven sampling sites.

Although emissions were typically dominated by indoor sources, the average contribution of outdoor sources (via ventilation) to total sources was higher for fungi than for bacteria. Average ( $\pm$ COV) indoor fungal emissions accounted for 62 $\pm$ 28% of total indoor air

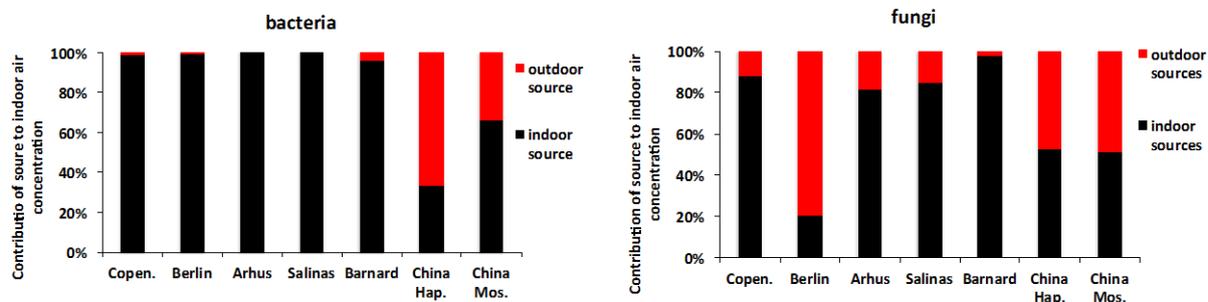


Figure 2. Comparison of the relative contributions of outdoor sources ( $Q \times C_{\text{out}}$ ) versus occupancy-associated indoor sources. Data represents the sum across all particle sizes.

contributions compared to  $85\pm 26\%$  for bacteria and  $79\pm 23\%$  for particulate matter (Figure 2). Regarding the variance of the indoor source strength versus outdoor sources with particle size distribution, the general trend in bacteria, fungi, and particulate matter was an increase in the relative strengths of the indoor sources with increasing particle size. In the 21 cases considered (seven locations for bacteria, fungi, and particulate matter mass), the fraction of indoor sources increased with particle size in all but two cases. The relative strength of outdoor sources was correlated to air exchange rate ( $r = 0.63, 0.64,$  and  $0.88$  for bacteria, fungi, and PM respectively).

## CONCLUSIONS

To understand how humans are exposed to beneficial and pathogenic microorganisms in indoor air, molecular-based microbial enumeration and identification must be integrated with investigations that assess the influence of building characteristics and aerosol physics-based processes. The results from this study provide quantitative information on indoor and outdoor bacterial, fungal, and total aerosol concentrations during periods of occupancy and vacancy, estimates of the relative contributions of indoor emissions to outdoor source contributions for bacteria and fungi, size-resolved emission rates attributable to human occupancy, and a quantitative comparison of the major sources of biological aerosols in indoor air. By combining size-resolved, molecular-based biological aerosol concentrations with building variables and material-balance models, this study provides important baseline data to inform quantitative approaches for understanding the sources of and human exposures to bacteria and fungi suspended in indoor air.

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