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Chamber bioaerosol study: human emissions of size-resolved fluorescent biological aerosol particles

Abstract Humans are a prominent source of airborne biological particles in occupied indoor spaces, but few studies have quantified human bioaerosol emissions. The chamber investigation reported here employs a fluorescence-based technique to evaluate bioaerosols with high temporal and particle size resolution. In a 75-m^3 chamber, occupant emission rates of coarse (2.5–10 μm) fluorescent biological aerosol particles (FBAPs) under seated, simulated office-work conditions averaged 0.9 $\pm$ 0.3 million particles per person-h. Walking was associated with a 5–6$\times$ increase in the emission rate. During both walking and sitting, 60–70% or more of emissions originated from the floor. The increase in emissions during walking (vs. while sitting) was mainly attributable to release of particles from the floor; the associated increased vigor of upper body movements also contributed. Clothing, or its frictional interaction with human skin, was demonstrated to be a source of coarse particles, and especially of the highly fluorescent fraction. Emission rates of FBAPs previously reported for lecture classes were well bounded by the experimental results obtained in this chamber study. In both settings, the size distribution of occupant FBAP emissions had a dominant mode in the 3–5 μm diameter range.

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Practical Implications

Biological particles, including skin squames and microbes, are ubiquitous in indoor air and constitute a substantial portion of coarse airborne particulate matter. Human emissions of fluorescent biological particles as measured in this chamber study were strongly associated with the vigor of activities. More than two-thirds of the emissions, on average, originated from the floor even when occupants were seated. Overall, results were quantitatively consistent with previously reported findings from observational monitoring in a university classroom. This study adds quantitative information to the emerging body of evidence that human occupancy contributes meaningfully to indoor bioaerosol levels.

Introduction

Bioaerosols have been linked to potential adverse health outcomes including infectious disease transmission, acute toxic effects, and respiratory symptoms of asthma and allergy (Douwes et al., 2003; Edwards et al., 2012). On the other hand, microbial exposures have also been associated with protective effects for atopic conditions (Ege et al., 2011; Fujimura et al., 2014; Yazdanbakhsh et al., 2002). In addition to human health effects, microbes indoors can contribute to negative esthetic impacts and to material damage. The presence and activities of humans have been found to substantially influence indoor bioaerosol concentra-

tions (e.g., Bartlett et al., 2004; Brandl et al., 2008; Chen and Hildemann, 2009; Clark and Shirley, 1973; Dechow et al., 1997; Fox et al., 2005; Lehtonen et al., 1993; Luoma and Batterman, 2001; Toivola et al., 2002). However, human bioaerosol emissions have been quantified in only a few studies. Characterizing emission rates is fundamentally important as emissions are expected to have greater generalizability than concentrations across varying indoor environmental conditions.

Previous studies quantifying human bioaerosol emissions can be classified into two groups: (i) controlled laboratory tests and (ii) observational assessments. A series of historical controlled studies from 1960 to 1980 showed that humans copiously shed bacteria-laden skin scales (or squames) into the air (Noble, 1975), approximately 10% of which have an aerodynamic diameter smaller than 10 μm (Mackintosh et al., 1978). For culturable bacteria, the rate of shedding was associated with activity level, gender, clothing, lipids on the skin surface, and the degree of colonization of key skin sites (Clark, 1974; Davies and Noble, 1962; Noble et al., 1976). Later investigations have included measurements of bacteria and fungi using either culture-based techniques applied to short-term samples (Scheff et al., 2000) or culture-independent, quantitative polymerase chain reaction (qPCR) applied to samples collected over multiple days (Hospodsky et al., 2014; Qian et al., 2012). While providing important information about overall emission rates, these observational assessments have not differentiated between emissions from shedding (i.e., from the body envelope including skin, hair, and clothing) vs. resuspension (i.e., from the floor and other contact surfaces). Prior studies also have had limited capability to resolve short-term dynamic processes affecting bioaerosol levels in occupied spaces.

To evaluate the influence of temporal variability on bioaerosol concentrations and emissions, Bhangar et al. (2014) conducted an observational classroom study. That study utilized a laser-induced fluorescence-based sampling method, embodied in an ultraviolet aerodynamic particle sizer (UV-APS). It was the first to apply the UV-APS to evaluate fluorescent particle dynamics in an occupied, indoor environment. In studies outdoors that have utilized the UV-APS, the outcome measure is termed 'fluorescent biological aerosol particles' or FBAPs. Atmospheric FBAPs have been interpreted as a lower limit estimate for primary biological aerosol particles. This measure excludes non-fluorescent biological materials, such as dead or dormant cells, but is not expected to contain significant non-biological interferents (Huffman et al., 2010; Pöhlker et al., 2012). Indoor FBAPs are expected to include human skin flakes (Bliznakova et al., 2007) and microbial cells (Hairston et al., 1997; O'Connor et al., 2011). The contribution of non-biological fluorescent materials to the indoor FBAP signal is not well known. Results from the classroom study (Bhangar et al., 2014) showed that occupants were a major source of airborne FBAPs, but did not allow for differentiation among contributing processes as activity conditions were uncontrolled. That study sampled from mixed and unresolved occupant activities, such as students seated taking notes, a professor pacing, and an occasional student walking in or out during lecture.

To evaluate the generalizability of the prior classroom findings and to probe the underlying processes, we undertook a set of experiments reported here. We employed an environmental chamber that allows for researcher control over independent parameters such as occupancy, activity, and air exchange rate. To differentiate between contributions from the body envelope vs. resuspension from flooring, separate experiments were conducted with the room floor exposed vs. covered with clean plastic sheeting. To evaluate the influence of the type and level of activity, different numbers of volunteer occupants followed scripted behaviors. This article characterizes the influence of occupancy, activity, and flooring conditions on human size-resolved FBAP emissions rates. The results are evaluated on a per-person basis and also normalized per mass of metabolic CO_2 emitted. A separate paper will report on the effects of occupancy, ventilation, and flooring on the microbial composition of airborne particles (RI Adams et al., Chamber bioaerosol study: Outdoor air and human occupants as sources of indoor airborne microbes, submitted to *PLoS ONE*).

Methods

Experimental design and sampling

The experiments were conducted in a controlled environmental chamber (Figure S1a) that simulates an office room. The room is situated on the second story on the southwest corner of a university building (Arens et al., 1991). Its physical dimensions of 5.5 m × 5.5 m × 2.5 m correspond to an interior volume of approximately 75 m^3. The flooring comprises 0.6 m × 0.6 m rubber-backed tiles, topped with closed-loop nylon carpeting. The tiles were approximately two years old at the time of this study. The chamber has large, inoperable, triple-glazed windows along its two exterior walls and is served by its own heating, ventilating, and air-conditioning (HVAC) system, which was installed in 1989. The HVAC system is configured to supply thermally and humidity conditioned outdoor air, without recirculation, that has passed through MERV-7 pleated filters. The air is cooled (with chilled-water coils) to a target set-point temperature (5–15°C) and then heated, first with a water-based system and finally with electrical resistance, to maintain the desired interior air tempera-

ture. For this study, the indoor temperature target was 22°C. Air was supplied to the room from an underfloor plenum through floor-level diffusers. A ceiling fan was operated on a low setting (approximately 50 revolutions per minute, blade diameter = 1.5 m, three blades) to maintain mixed conditions in the room. The steam humidifier in the HVAC system was off, except for a few cases in which relative humidity (RH) was manipulated as a study variable.

The effects of varying human occupancy and activity conditions were evaluated through 47 two-hour experiments (also referred to here as 'treatments' or Tm). The first set of 30 'basic' treatments was conducted in December 2013; 17 supplementary treatments were conducted in January 2014. The basic and supplementary experiments were followed by two additional treatments, conducted in June 2014, to assess the microbial composition of supply air. Protocols for recruiting human subjects and study procedures were reviewed and approved by the Committee for the Protection of Human Subjects at the University of California, Berkeley (Protocol ID 2013-01-4927).

The basic treatments were designed to examine the contribution of particle resuspension from the carpeted floor and the influence of different levels of occupancy and activity. The carpet was tested in one of two states: floor exposed (exp) or covered with clean plastic sheeting (cov). Five states of occupancy and activity were investigated: unoccupied, one person seated, two people seated, eight people seated, and two people walking. As summarized in Table 1a, these conditions were tested with triple replication for a total of 30 experiments ($2\times5\times3$). (For one eight-person treatment, only six subjects were available, so we multiplied emission rates by 1.33 (8/6) for that one experiment.) The order of occupancy-and-activity level was randomized within each flooring condition. Randomizing across the floor configurations was impractical, so exposed vs. covered floor conditions were evaluated during contiguous weeklong study periods.

Supplementary treatments were designed to explore the potential influence of additional variables including intensity of upper body movements, quantity of clothing, and elevated relative humidity (Table 1b). To reduce the contribution of outdoor air and to allow us to more clearly observe the influence of indoor activities on indoor concentrations, eight of 17 supplementary treatments were conducted with reduced HVAC fan speeds that corresponded to reduced air exchange rates. Treatments conducted in June, as part of the post-experiment evaluation of the microbial composition of supply air, are also summarized in Table 1b.

During all treatments, either the exposed floor was vacuumed daily or the plastic on the covered floor (1 mm polyethylene, Polar Plastics #5680000; purchased through Grainger Inc, San Leandro, CA, USA) was replaced daily. These maintenance activities were conducted either at the end of the day after treatments were concluded or in the morning at least 45 min before treatments commenced. During floor-covered walking treatments, clean antistatic strips (3.5-mm vinyl table mat, All-Spec Industries #8264, Wilmington, NC, USA) were laid on top of the plastic to avoid influence from electrostatic charge.

Demographic information for the volunteer subjects is as follows. Two key female volunteers, referred to as V1 and V2, participated in many basic treatments and in all the supplementary treatments. Only female volunteers participated in walking treatments. In contrast, basic seated treatments included substantial proportions of male subjects (11 of 31 and 13 of 33 for the floor-exposed and floor-covered configurations, respectively) as indicated in Table 1a. These experiments were not designed to investigate the influence of gender on emission rates.

During all treatments, indoor sampling was conducted from a location in the core of the room, at a height of 1.1 m, which approximates the breathing height of a seated occupant. Outdoor sampling was conducted through a window from a room near the chamber that was alongside the HVAC system air intake (Figure S1b). A UV-APS (model 3314, TSI, Inc., Shoreview, MN, USA) measured indoor particle levels in 51 size and 64 fluorescence intensity bins, for particles with aerodynamic diameters in the range 0.5–20 μm, and with 5-min resolution. The configuration of the device and its placement in an air-cooled enclosure are described in Bhangar et al. (2014). Supplementary particle and copollutant monitors were used to simultaneously collect additional, time-resolved (once every minute) data, both indoors and outdoors, during each treatment. These monitors included optical particle counters (OPC; model GT-526, Met One, Inc., Grants Pass, OR, USA), which measured concentrations of particles larger than 0.3 μm, resolved into 6 size groups. We also utilized temperature and relative humidity monitors (HOBO U10-003; Onset Computer Corp., Bourne, MA, USA) and carbon dioxide monitors (model 820, LI-COR Biosciences, Lincoln, NE, USA). Outdoor particle data are missing from treatments 10–12 because of instrument malfunctioning. During a subset of treatments (20 of 49), total particle number (PN) concentrations were sampled at a second peripheral indoor location to evaluate mixing conditions in the room.

The mean temperature and relative humidity sampled indoors, outdoors, and in the supply air plenum are summarized in Table 1a,b and are discussed in the Appendix S1.

Data interpretation

Particle counts recorded by the UV-APS were processed by means of evaluating size-resolved number

Table 1 Schedule of (a) basic and (b) supplementary treatments (Tm) along with mean values of environmental data acquired indoors (*i*), outdoors (*o*), and in the underfloor supply air plenum (s) during the second hour of each experiment[a,b,d,e]

			Occupancy								
Tm	Date and time (2013)[c]	Activity	M	F	Floor	T_i (°C)	RH_i (%)	T_s (°C)	RH_s (%)	T_o (°C)	RH_o (%)
(a)											
1	12/09 10:07	sit		2	exp	22	≤15	22	≤15	9	18
2	12/09 13:10	sit	2	4	exp	22	≤15	20	≤15	11	≤15
3	12/09 16:05	unoc			exp	21	≤15	24	≤15	10	≤15
4	12/10 10:03	sit	3	5	exp	23	20	20	17	12	31
5	12/10 13:01	walk		2	exp	22	≤15	21	≤15	14	26
6	12/10 15:55	sit		1	exp	22	≤15	23	≤15	12	29
7	12/11 10:01	unoc			exp	22	≤15	23	≤15	14	24
8	12/11 13:02	sit	5	3	exp	23	18	20	17	15	27
9	12/11 15:57	sit	1	1	exp	22	≤15	21	≤15	14	30
10	12/12 10:01	walk		2	exp	22	27	21	30	15	45
11	12/12 13:02	sit		2	exp	22	28	21	30	16	41
12	12/12 16:00	sit		1	exp	22	28	23	28	14	46
13	12/13 10:01	sit		1	exp	22	32	22	35	14	58
14	12/13 13:00	walk		2	exp	22	34	20	39	15	53
15	12/13 15:50	unoc			exp	22	33	23	31	14	48
16	12/16 09:51	sit		2	cov	22	28	22	28	18	36
17	12/16 13:01	sit	5	3	cov	23	29	20	30	20	29
18	12/16 15:55	unoc			cov	22	26	21	28	18	33
19	12/17 10:00	sit	3	5	cov	24	32	20	34	17	41
20	12/17 12:59	sit		1	cov	22	33	20	38	19	42
21	12/17 16:01	walk		2	cov	22	35	21	36	16	48
22	12/18 10:00	unoc			cov	22	31	23	31	15	55
23	12/18 13:00	sit	5	3	cov	24	36	20	41	15	63
24	12/18 16:33	sit		2	cov	22	36	22	36	12	75
25	12/19 10:12	walk		2	cov	22	28	21	28	15	39
26	12/19 13:01	sit		2	cov	22	25	21	24	17	30
27	12/19 16:02	sit		1	cov	22	≤15	23	≤15	15	22
28	12/20 10:01	sit		1	cov	22	≤15	22	≤15	15	22
29	12/20 13:02	walk		2	cov	22	≤15	20	≤15	17	21
30	12/20 15:50	unoc			cov	22	18	23	20	14	35

Tm	Date and time (2014)[c]	Activity	Floor	*a*	Clothing	Movement	Humidity	T_i (°C)	RH_i (%)	T_s (°C)	RH_s (%)	T_o (°C)	RH_o (%)
(b)													
31	1/07 10:28	walk	cov	med	red			24	30	19	39	14	51
32	1/07 14:00	walk	cov	med	tyv			24	30	18	41	16	47
33	1/08 10:02	sit	exp	low				23	34	18	43	14	70
34	1/08 14:07	unoc	exp	low				22	33	19	42	14	77
35	1/09 10:10	sit	exp	low				23	34	18	43	15	67
36	1/09 14:09	sit	exp	low		low		23	33	18	42	17	50
37	1/10 10:13	sit	exp	low		high		23	34	18	44	15	64
38	1/10 14:08	unoc	exp	low				23	32	18	42	18	51
39	1/13 10:03	sit	exp					23	24	20	27	17	33
40	1/13 13:07	walk	exp					23	22	20	25	20	25
41	1/13 16:00	unoc	exp					22	21	20	26	18	31
42	1/14 10:03	walk	exp				40%	24	46	21	62	18	31
43	1/14 13:03	sit	exp				40%	24	43	21	49	21	26
44	1/14 15:58	unoc	exp				40%	23	43	21	49	19	28
45	1/15 10:55	unoc	exp				80%	25	74	21	96	22	≤15
46	1/16 10:10	sit	exp				80%	26	70	23	98	22	17
47	1/16 13:00	walk	exp				80%	25	74	21	101	23	≤15
48	6/20 09:58	walk	exp	med				23	35	[f]	[f]	21	56
49	6/20 12:51	unoc	exp	med				22	34	[f]	[f]	24	52

[a]The relative humidity sensors had a lower limit of measurement of 15%.

[b]All basic treatments were conducted with the floor exposed (exp) or covered with clean plastic sheeting (cov) and no humidity control. Occupants (M = male, F = female) wore regular clothes (of their own choice) and were either sitting (sit), walking (walk), or absent (unoc). During seated treatments, occupants were engaged in normal desk work (usually with laptop computers).

[c]Experiment start times are indicated. Each experiment lasted 2 h.

[d]The following parameters were varied during the supplementary treatments as indicated: two female occupants sitting (sit), walking (walk), or absent (unoc); floor covered (cov) or exposed (exp); air exchange rate (*a*) medium (med) or low; clothing reduced (red) or covered with Tyvek (tyv); movement of the upper body high or low; humidity control (set point 40% or 80%). Blank cells indicate 'normal' conditions that matched those for the basic treatments.

[e]Treatments 48 and 49 were conducted to evaluate the microbial composition of supply air. Results from those treatments are discussed in a separate paper on microbial community composition.

[f]Missing data.

concentrations in the 1–10 μm size range. Particles were further sorted into three categories according to fluorescence intensity: total particles (with number concentration N_T having units of per cm^3), fluorescence intensity >2 (referred to as FBAPs, with number concentration symbolized by N_F, per cm^3), and fluorescence intensity >20 (referred to as highly fluorescent particles, with the number concentration symbolized by N_{F20}, per cm^3). The rationale for choosing these fluorescence intensity thresholds is discussed by Bhangar et al. (2014) (see also Figure S2).

Size-integrated 'coarse' particles were defined to have a lower diameter limit of 2.5 μm, and upper limit of 10 μm, to match the conventional and regulatory sorting of aerosol particles into $PM_{2.5}$ and PM_{10}, and also because our analysis was robust only within these limits. For particles smaller than 2.5 μm, emissions attributable to occupancy were uncertain because ventilation was a dominant, variable, and incompletely characterized source. For particles larger than 10 μm, estimates were imprecise due to small absolute particle number concentrations that were close to the detection limit of the sampling method. Size-integrated results from a previous study (Bhangar et al., 2014), which included particles in the 1–15 μm diameter range, have been reassessed to match the 2.5–10 μm range when reported in the present paper.

The particle and carbon dioxide profiles during each treatment followed the expected pattern of high concentrations during the transition period when subjects entered the room, followed by a gradual relaxation to quasi-steady state conditions as subjects settled into each prescribed activity. Consequently, a steady state approximation was invoked to evaluate emission rates. Mean concentrations measured during the second hour of each two-hour experiment were considered as representative of the prescribed activity and used as inputs in the analysis.

Occupant emission rates (ER_{ij}, in units of particles/h) were estimated for the combined processes of shedding and resuspension for each basic and supplementary treatment j. The emission rates were assessed for FBAPs and for highly fluorescent particles utilizing seven lumped particle size groups i, which were distilled by grouping the 32 UV-APS size bins between 1 and 10 μm; see Table S1. Emission rates were evaluated using a steady state approximation of a single-compartment material balance model (Equation 1) following the method used to estimate occupant FBAP emission rates in a classroom (Bhangar et al., 2014).

$$ER_{ij} = N_{net,avg,ij} \times V \times (k_i + a) \quad (1)$$

In Equation 1, $N_{net,avg,ij}$ is the mean increment in particle number concentration attributable to occupants (particles per cm^3), V is the interior chamber volume (cm^3), k_i is the first-order particle deposition loss rate coefficient (per h), and a is the air exchange rate (per h). $N_{net,avg,ij}$ was evaluated as the mean indoor N_F or N_{F20} concentration, determined for steady state conditions, minus the baseline. The baseline represented the sum of the contribution from outdoors and from the HVAC system. It was estimated as the average of the 10-min mean indoor concentrations measured twice: (i) before each treatment was initiated and (ii) starting ~45 min after the treatment was concluded, with the delay designed to allow particles generated during the treatment to be flushed from the room. The 45-min period constitutes about two air changes at the normal HVAC settings. For the last treatment in each set (i.e., Tm30 and Tm47), a 'post'-baseline was not available because chamber maintenance was initiated immediately following the end of a treatment. In these two cases, the baseline was based only on measurements before each treatment was initiated. We assumed throughout this analysis that the chamber was well mixed based on the observation that PN concentrations (of particles larger than 2 μm) at two locations in the room consistently agreed to within 20% (average difference = 10%).

Air exchange rate assessments relied on metabolically generated CO_2 as a tracer gas. The air exchange rate, a, was evaluated as the exponential decay of the net (indoor minus outdoor) CO_2 mixing ratio during the immediate post-treatment period when the room experienced the abrupt change-of-state from being occupied to being unoccupied. These assessments were made for each treatment, excluding zero-person treatments and a few others; see Table S2. Mean ($\pm$ standard deviation) values of a corresponding to the normal, medium, and low fan speeds were 2.8 $\pm$ 0.2/h (N = 20), 1.9/h (N = 2) and 1.4 $\pm$ 0.1/h (N = 4), respectively.

Previously published (Thatcher et al., 2002), size-resolved deposition loss rate coefficients (k) that matched the mean airspeed condition in the core of the chamber (14 cm/s) were employed in the analysis. Empirical k_i estimates were also evaluated, on the basis of net N_F decay rates during the immediate post-emission period, following the approach described in Bhangar et al. (2014). Published estimates from Thatcher et al. (2002) were considered more reliable as the empirical estimates were few and noisy, mainly because net N_F levels in the room at the end of each treatment were too low to consistently support robust calculations. Hence, the new empirical evidence is used only to substantiate that the Thatcher et al. (2002) results were appropriate to apply in this study, as illustrated in Figure S3.

The occupancy-associated emission rate of total particles could not be accurately determined by applying the same approach with N_T as was implemented for N_F, because of the pronounced influence of out-

door levels on indoor concentrations, combined with the greater variability of outdoor total particle concentrations. Instead, total particle emission rates were evaluated by applying Equation 1, with $N_{net,avg,ij}$ evaluated as the mean indoor PN concentration minus the product of the simultaneous mean outdoor PN concentration and the expected indoor proportion of outdoor particles, or *IPOP*. In this assessment, we utilized data from the optical particle counters. A mean value for *IPOP* was evaluated per particle size bin, as the average indoor PN concentration, divided by the average outdoor PN concentration, during zero occupancy basic treatments ($N = 6$). A separate *IPOP* was assessed for reduced fan speed tests ($N = 2$). (Table S3). (The *IPOP* parameter (Riley et al., 2002) is also referred to in the literature as the 'infiltration factor' (Chen and Zhao, 2011).) The use of a different analysis approach and particle size measurement technique for determining FBAP and total particle emission rates limits their intercomparability.

Metabolic carbon dioxide emission rates (g/h) during each treatment were also evaluated using Equation 1, with the average net indoor CO_2 level assessed as the difference between indoor and outdoor levels during each treatment, and with $k_i = 0$. The evaluated carbon dioxide emission rates were used to normalize FBAP emission estimates. This normalization reduces uncertainties associated with assumptions that the room was well mixed and that the air exchange rate was invariant with time. The normalization also dampens the influence of human activity intensity on emissions, as more vigorous activities were associated with a higher metabolic CO_2 emissions rate as well as with higher coarse particle emissions.

Quality control

The UV-APS size (Figure S4) and fluorescence responses were found to be within manufacturer specifications as assessed by testing with monodispersed polystyrene latex (PSL) particles with physical diameters in the range 0.6–2 μm (Duke Scientific Corp., Fremont, CA, USA; Polysciences, Inc., Warrington, PA, USA). To calibrate optical particle counters (OPCs), each unit was tested a minimum of twice, once before and once after the treatments, alongside a reference device (OPC3) for at least 1.5 h in the chamber or laboratory. Table S4 summarizes slopes or

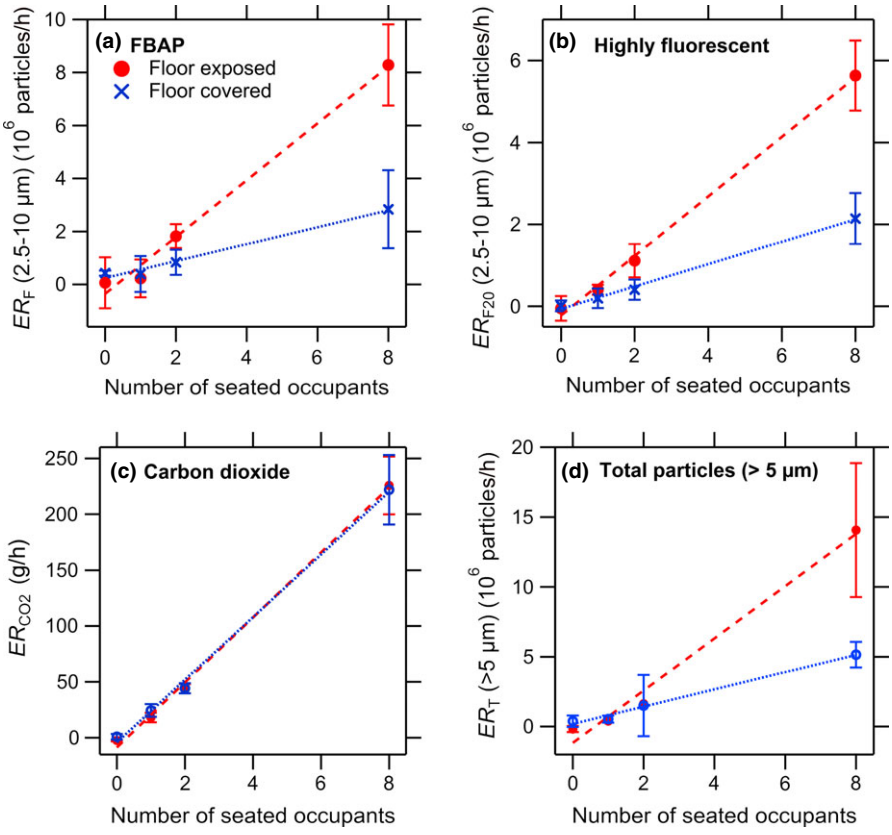

Fig. 1 Emission rates associated with normal desk work: mean (± standard deviation) emission rates (*ER*) of (a) coarse (2.5–10 μm) fluorescent biological aerosol particle numbers, (b) coarse highly fluorescent particle numbers, (c) carbon dioxide mass, and (d) numbers of total particles larger than 5 μm. These are the results from basic treatments, when varying numbers of occupants (0, 1, 2, 8) were seated engaged in normal desk work, with the carpeted floor exposed or the floor covered with clean plastic sheeting. Error bars show standard deviations. A best-fit line (least-squares linear regression) for each group of results is shown

'adjustment factors' derived from the calibration tests. Accurate responses (to within the manufacturer specified '3% of reading') of carbon dioxide monitors were confirmed using calibration gases at 0 and 1000 ppm.

Results and discussion

Emissions from seated occupants

Figure 1 shows emission rates for conditions when occupants were seated and engaged in normal desk work (including use of laptop computers). The mean emission rates of size-integrated (2.5–10 μm) fluorescent (ER_F) and highly fluorescent (ER_{F20}) coarse particle numbers, size-integrated (>5 μm) total particle numbers (ER_T), and carbon dioxide mass (ER_{CO2}) were linearly correlated with the number of occupants, with high coefficients of determination ($r^2 > 0.98$). The slope of the regression line between particle emission rates and the number of seated occupants was significantly lower when the floor was covered with a clean plastic sheet as compared with the exposed floor condition, confirming that resuspension from the carpet was a strong source of coarse particles regardless of fluorescence intensity and even when occupants were seated.

Figure 1 presents results from basic treatments. Estimates from unoccupied periods (occupancy = 0) are intended as controls. A particle size threshold of 5 μm was chosen for evaluating total occupancy-associated particle emissions because total particle results for smaller size groups were not robust (Figure S5).

Weighted mean ± standard deviation per person-h emission rates for the index condition—occupants seated and floor exposed—during basic treatments was 0.9 ± 0.3 million coarse FBAP numbers, 1.6 ± 0.7 million total number of particles >5 μm, and 26 ± 5 gCO_2 (Table 2). Highly fluorescent particles constituted about two-thirds of the weighted-average FBAP emissions for seated occupants under both flooring conditions. Weighting was applied to adjust for the uneven number of subjects included in each seated treatment, as we have greater confidence in estimates from treatments with a larger number of subjects because observed concentrations were farther above the baseline. The estimated mass emission rates of coarse FBAP (and total particles larger than 5 μm) were approximately 0.06–0.2 (0.2–0.6) mg per person-h. About 75% of the FBAP mass was in particles larger than 5 μm in diameter. Mass emission rates were assessed based on the assumptions that particles were spherical, had a density in the range of 1 to 2.5 g/cm^3 (Ferro et al., 2004a), and that, within each lumped particle size group, the mass-weighted size distribution, $dM/d(\log d_a)$, was constant.

Figure 2 presents size distributions of normalized (per g of CO_2 emitted) weighted mean (±s.d.) FBAP emission rates (E_F) when occupants were seated, with the floor exposed or covered, during basic treatments. The size mode of FBAP emitted by seated occupants was approximately 3 μm; this measure did not change significantly for floor-covered conditions. Figure S6 shows that the size mode of highly fluorescent particle emissions was slightly larger (about 4 μm).

FBAP and total particle emission rates for treatments conducted during the supplementary period were lower than obtained on average for the basic set (Table 2). When normalized by CO_2 emission rates, the basic and supplementary 'seated' estimates had similar magnitudes and size distributions (Figure S7). However, differences between basic and supplementary period 'walking' emission rates were evident even in the CO_2-normalized results (Figure S8). The limited number of experiments is a barrier to determining the cause; one possible factor could be a change in the floor reservoir of material available for resuspension owing to shifts in occupancy patterns and cleaning practices during the intervening month between the basic and supplementary treatments.

Table 2 Summary of mean ± standard deviation per-person emission rates of coarse fluorescent biological aerosol particle (FBAP) numbers (ER_F), numbers of total particles larger than 5 μm (ER_T), carbon dioxide mass (ER_{CO2}), and the fraction of emitted FBAPs that were highly fluorescent (ER_{F20}/ER_F) as a function of occupancy conditions and floor configuration (cov = covered, exp = carpeted floor exposed)

Occupancy condition	Floor	ER_F (10^6 per person-h)	ER_T (10^6 per person-h)	ER_{CO2} (gCO_2 per person-h)	ER_{F20}/ER_F	N[a]
Basic						
Seated	exp	0.9 ± 0.3	1.6 ± 0.7	26 ± 5	0.69	9
Seated	cov	0.4 ± 0.2	0.7 ± 0.4	26 ± 4	0.67	9
Walking	exp	6 ± 1	9 ± 4	38 ± 1	0.54	3
Walking	cov	1.7 ± 0.5	3 ± 1	39 ± 2	0.58	3
Supplementary						
Seated	exp	0.53 ± 0.07	0.8 ± 0.3	19 ± 1	0.76	3
Walking	exp	3.5	6.4	38	0.64	1
Seated, low movements	exp	0.05	0.2	18	ND[b]	1
Seated, high movement	exp	2.4	4.6	19	0.72	1
Swimsuit walk	cov	0.8	1.3	34	0.27	1
Tyvek walk	cov	1.9	4.4	35	0.35	1
Classroom[c]						
Lectures	exp	0.9 ± 0.4	n/a	18 ± 3	0.53	49

[a]Sample size (i.e., number of replicate treatments). For total particle floor-exposed means: $N = 7$ for seated treatments and $N = 2$ for walking treatments, because outdoor PN concentration data were missing for Tm10–Tm12.

[b]Not determinable. Emissions were below the limit of quantification leading to an ER_{F20}/ER_F ratio >1, which lacks physical meaning.

[c]From Bhangar et al. (2014), with ER_F and ER_{F20} re-assessed to match the 2.5–10 μm range reported in the present paper. The classroom had a hard tiled floor that was cleaned daily. The mean occupancy load during each lecture ranged from 21 to 80 adults.

The influence of activity level

Figure 3 presents size distributions of normalized FBAP emissions for walking with the floor exposed or covered during basic treatments. Compared with sit-

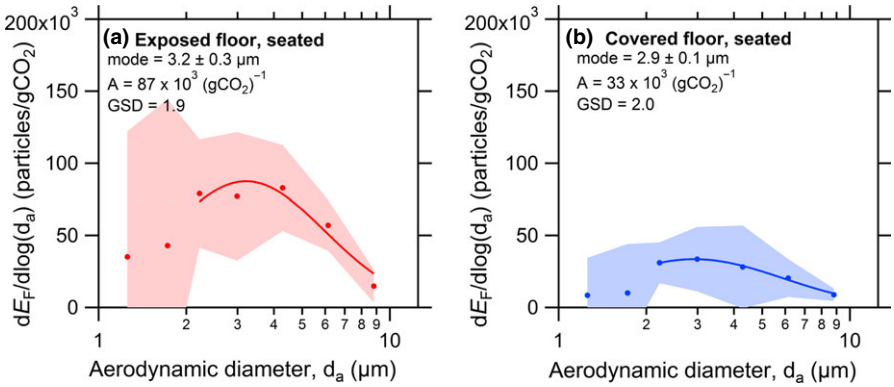

Fig. 2 Size distributions of weighted mean (± standard deviation) occupant emissions of fluorescent biological aerosol particle numbers (E_F) during basic treatments when 1–8 occupants were sitting engaged in normal desk work. Emissions are normalized by the mass of carbon dioxide emitted and shown as a function of particle aerodynamic diameter (d_a). During the treatments, the carpeted floor was (a) exposed or (b) covered with clean plastic sheeting. There were 9 treatments and 31–33 subjects per floor condition. The mode, geometric standard deviation (GSD), and amplitude (A) of lognormal distributions that provide the best fit to emissions of particles larger than 2 μm are shown on each panel

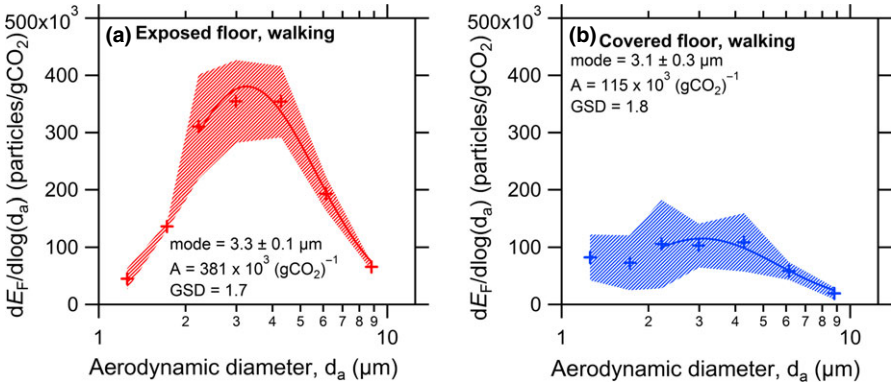

Fig. 3 Size distributions of weighted mean (± standard deviation) occupant emissions of fluorescent biological aerosol particle numbers (E_F) during basic treatments when 2 occupants were walking in the chamber. Emissions are normalized by the mass of carbon dioxide emitted and shown as a function of particle aerodynamic diameter (d_a). During the treatments, the carpeted floor was (a) exposed or (b) covered with clean plastic sheeting ($N = 3$ treatments per floor condition). The mode, geometric standard deviation (GSD), and amplitude (A) of lognormal distributions that provide the best fit to emissions of particles larger than 2 μm are shown on each panel

ting, these results show that walking was associated with much higher emission rates, but with a similar FBAP emission size mode. Walking was expected to increase particle emissions relative to sitting because of enhanced resuspension (from increased intensity of contact with the floor), and because of an increase in the shedding rate (from increased vigor of bodily movements). Results showed that the combined effect of these two processes, when the floor was exposed, led to per-person walking/sitting ER_F (ER_T) ratios of 6.1 (5.8) (Table 2). When the floor was covered, the walking/sitting ER_F (ER_T) ratios were moderately smaller, 4.7 (5.3). These results are qualitatively consistent with prior research showing that bioaerosol concentrations are elevated under high-activity conditions, for instance during boarding and deplaning as compared to cruise conditions on a flight (McKernan et al., 2008). Similar scales of increase of human particle emissions associated with activity have been seen in other quantitative studies conducted for total particles (Ferro et al., 2004b; You et al., 2013).

Apart from the magnitude of the particle emission rate, two FBAP signature elements showed a characteristic pattern for walking compared to sitting. First, the mean minimum fractions of coarse FBAP emissions that were inferred to originate from the floor (i.e., the fraction reduced by covering the floor) were 61% during sitting and 70% during walking. One might expect resuspension from the floor to be greater when occupants are walking than sitting. However, the scale of the difference observed here was smaller than we had anticipated. Second, on average, within each set of treatments (basic or supplementary), a smaller fraction of the mean FBAP emitted was highly fluorescent for walking as compared with sitting (Table 2). This difference suggests that the highly fluorescent particles were

present in greater proportions in the aerosol particles shed from the human envelope (either directly or through contact with non-floor surfaces) than among the particles resuspended from the floor. However, this finding is only suggestive, as differences in ER_{F20}/ER_F ratios across the basic vs. supplementary treatment periods limit the utility of this metric as a more quantitative source indicator.

In addition to walking, a second high-intensity activity was tested: enhanced upper body movement. In a pair of supplementary treatments, occupants were instructed to either sit as still as possible (Tm36) or move their upper bodies while holding constant the contact with the exposed floor (Tm37). Enhanced movements included a choreographed series of common indoor activities: normal computer work (index condition), brushing hair, taking outer-wear on and off, flipping pages of a book, stretching, and rubbing face and arms. Results of these two supplementary tests showed that increased upper body movements were associated with an approximately 5× higher ER_F and ER_T than the average for supplementary 'regular' seated treatments (Table 2). A time series of N_F, N_{F20}, and N_F/N_T in the room during these activities (Figure S9) shows that the highest FBAP concentrations were associated with taking outer clothing on and off, although elevated levels were observed during each of the five manipulations, as compared to the index condition of computer work.

Emission rates of FBAPs and total particle numbers (but not CO_2) were barely discernible when occupants minimized movements. The strong reduction in emissions when occupants minimized movements suggests that total bioaerosol emissions during even quiet wakeful activities are dominated by particles dislodged due to movement. Only a small minority, if any, is passively emitted, for example, from the respiratory tract during breathing, or shed from the body through passively generated airflow. Although the respiratory tract is implicated as a source of specific biological agents (e.g., Nicas et al., 2005), previous research confirms that breathing is not expected to emit substantial numbers of coarse particles compared to skin and clothing. For example, Holmgren et al. (2010) and Morawska et al. (2009) showed that the size distributions of particles emitted during breathing have a peak in the submicron size range, with particles larger than 2.5 μm emitted in substantial quantities only during sustained vocalization.

Preliminary explorations: the influence of clothing and relative humidity

Clothing was observed to be an important potential source in the treatment with enhanced upper body movements. Therefore, the influence of clothing was explored through a second series of supplementary manipulations conducted with the floor covered to focus on emissions by shedding. In a pair of walking treatments, clothing was either minimized (subjects wore swimsuits and had bare feet, Tm31), or subjects were covered with a Tyvek suit and also wore N95 filtering respirators, hoods, and booties (Tm32). Tyvek has been found to have a very low penetration for airborne culturable bacteria (Lidwell et al., 1978). As is evident in the results presented in Table 2, covering clothes with Tyvek did not exhibit a strong influence on ER_F or ER_T. On the other hand, reduced clothing did result in moderately reduced FBAP and total particle emissions, and a strongly reduced ER_{F20}/ER_F ratio. These results suggest that the highly fluorescent particles may be preferentially associated with clothing or shoes. Further research is warranted to evaluate whether the highly fluorescent particle signal includes contributions from fluorescent whitening agents added to clothing and detergents as was suggested as a possibility in Bhangar et al. (2014).

Previous research suggests that clothing may increase bioaerosol emissions by indirect or direct processes. Indirectly, clothing may be a source by promoting the detachment of bacteria-laden skin flakes via friction (Charnley and Eftekhar, 1969; Doig, 1972; Hall et al., 1986). Or, clothing may serve as a direct source by emitting particles previously deposited when the wearer engages in physical activity (McDonagh and Byrne, 2014a,b). Noble (1975) estimated that 5 mg of skin squames is deposited in clothing every hour, and Adams et al. (2013) showed that human surfaces act as passive samplers for environmental microbes. On the other hand, clothing has also been demonstrated to act as a particle sink that reduces shedding (Spendlove and Fannin, 1983). Additional research is needed to better understand the influence of clothing on total and fluorescent coarse particle emissions under a range of typical conditions.

Results from RH manipulations are discussed in Appendix S2.

Experimental error and interhuman variability

The variability in mean per-person emission rates between replicate treatments can be attributed to the combined influence of experimental error and differences among subjects and/or between testing days for the same subject. The expected influence of experimental error is revealed in the range of zero-occupant results. The range of 'control' results for FBAPs suggests that emissions associated with a treatment were quantifiable to about ± 1 million FBAP (2.5–10 μm) or total particle (>5 μm) numbers per hour (see Figure 1). For highly fluorescent particles, smaller differences (~0.3 million particles per h) in absolute emissions could be clearly resolved. The main source of experimental noise is likely the temporal variability of outdoor concentrations leading to corresponding

changes in the indoor concentration of particles of outdoor (or supply air) origin, between baseline and treatment periods (see Figure S10).

Differences among subjects could not be resolved in our study due to the low number of replicates of the same treatment with similar and dissimilar groups of subjects. Previous research has shown that men emit more particles containing culturable bacteria than women (Noble, 1975) and that some individuals may be super shedders (Hall et al., 1986; Mackintosh et al., 1978). Whether variability among subjects will prove to be substantial for FBAP emission rates is a topic for future investigations. We note that our CO_2 emission results showed that one of the primary volunteers, V1, had a systematically lower metabolic CO_2 emission rate than did the other subjects. Her mean 'seated' emission rate was 17 ± 2 gCO_2 per h (N = 3), considerably lower than the per subject average of 27 ± 3 gCO_2 per h (N = 5) for seated treatments when subject V1 was absent. Note, too, that the metabolic CO_2 emission rate of subject V1 was lower than the expected value of 22 gCO_2 per h based on height, weight, and gender (Qi et al., 2014). Subject V1 constituted 50% of the subject pool during the supplementary period, but only 20% during the basic period, plausibly accounting for a systematic difference in mean CO_2 emission rates between the two periods.

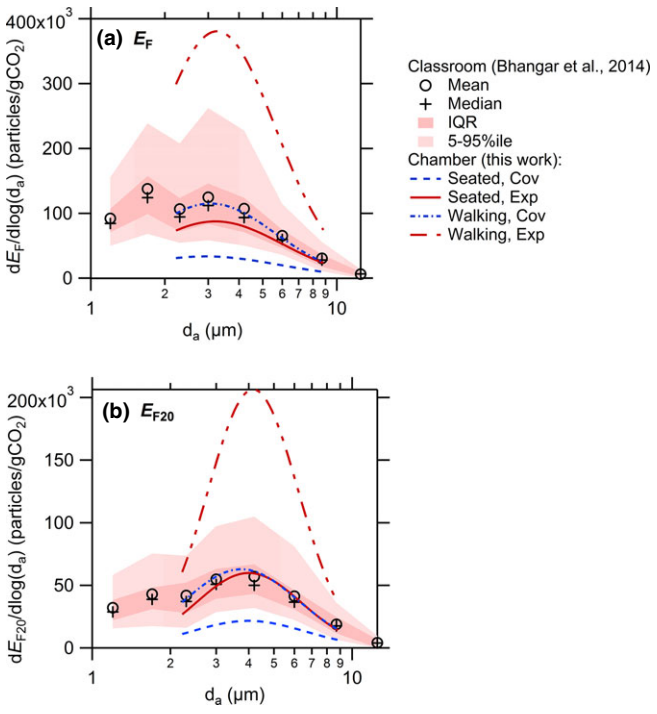

Fig. 4 A comparison between normalized fluorescent biological aerosol particle emissions (particles per gCO_2) evaluated under observational conditions during lecture class periods in a university classroom (Bhangar et al., 2014) and during controlled conditions in an environmental chamber (this study). The magnitude and size dependence of the central tendency of normalized estimates from the classroom were similar to mean emissions evaluated in the chamber when people were walking and the floor was covered, or when people were seated and the floor was exposed.

New results in context: A comparison with observational studies in classrooms

Figure 4 compares size distributions of mean normalized emission rates corresponding to basic treatments (from Figures 2 and 3) with the distribution of emission rates obtained under observational conditions in a classroom (Bhangar et al., 2014). The distribution of per-person emission rates obtained during lecture classes is well bounded by the experimental results from these chamber experiments. On average, classroom per-person emission rates (Table 2) were similar to chamber emission rates under the conditions that most closely matched lecture class sessions: occupants seated and engaged in normal desk work with the floor exposed. In both settings, the emitted FBAP size distributions had a dominant mode in the 3–5 μm range that has previously been observed in the atmosphere and was postulated to be fungal spores and agglomerated bacteria (Huffman et al., 2010). Also, in both settings, the fraction of total indoor airborne particles that was fluorescent increased with particle size and with the presence and activities of occupants.

A notable difference between the chamber and classroom studies was the extent to which outdoor particles contributed to indoor levels. Both rooms were served with outdoor air delivered by mechanical ventilation systems without recirculation. However, the classroom had better filtration, and, correspondingly, we found in the chamber study that indoor particle levels were more strongly influenced by outdoor changes than in the classroom. The relative strength of outdoor air as a source may be even greater in indoor environments with natural ventilation (Meadow et al., 2014), or in places and at times with prominent outdoor bioaerosol sources such as near heavy vegetation and during rainstorms (Bowers et al., 2013; Huffman et al., 2013). The occupant emission rates presented in this article have the merit of being applicable regardless of outdoor source and building ventilation characteristics.

Estimated human emission rates of total particles in the chamber were lower than previous estimates from seven classroom sites distributed globally (Hospodsky et al., 2014; Qian et al., 2012). Although the chamber results are based on particles larger than 5 μm, and the classroom results were for total suspended particulate matter, the two sets of results are compared here because the classroom emission size distributions showed that most of the emitted particle mass was associated with particles >5 μm in diameter. We focus on results from a classroom in Salinas, California, which was geographically closest to the chamber. The

human total particle emission rate in that classroom, under mixed activity conditions and averaged over a multiday period, was 8 mg/person-h. Average results from our chamber study were more than an order of magnitude lower during seated conditions (0.2–0.6 mg/person-h) and somewhat lower even when activity levels were high (occupants walking: 1–4 mg/person-h). Further research would be needed to allocate the difference in emission rates between our study and previous classroom investigations to the environment (i.e., to the floor reservoir available for resuspension), to human occupants (i.e., to differences in shedding from clothing and body surfaces), or to activities other than sitting and walking.

The bioaerosol emission rates in the Salinas classroom were 0.8 million bacterial cells per person-h and 4.7 million fungal spore equivalents per person-h (Hospodsky et al., 2014). The sum of bacterial and fungal emissions was higher than the mean coarse FBAP emission rate in the chamber under seated conditions and similar to the walking emission rate (Table 2). The emission size modes reported by Qian et al. (2012) and Hospodsky et al. (2014) were similar to those found in the present study. Although this comparison suggests that FBAP-based and qPCR-based results may be quantitatively comparable, the relationship needs further investigation and is not easily interpreted. Bacterial cells and fungal spore equivalents may be numerically greater than the number of airborne particles carrying fluorescent microbes for a few reasons: (i) because of the presence of multiple cells or spores per fluorescent particle (Noble and Davies, 1965; Tham and Zuraimi, 2005) and (ii) because dead/inactive cells with amplifiable genomic material may not fluoresce (Agranovski et al., 2003; Saari et al., 2014). On the other hand, FBAP numbers can be enhanced by the fluorescence of non-microbial components of indoor aerosol particles, such as skin flakes, and by non-biological fluorescent interferents (Bhangar et al., 2014). While it has been suggested that most of the airborne bacteria associated with particles larger than 2 μm are carried on desquamated skin (Clark, 1974; Fox et al., 2008), the proportion of skin-bearing fluorescent particles that lack microbes is not known.

Comparing FBAP emission rates (Figure 4, Table 2) and previously reported culture-based bioaerosol emission rates shows that the differences between these two outcome metrics may exceed the ranges attributable to occupancy-related and environment-related factors. Specifically, Scheff et al. (2000) estimated emission factors for colony-forming units (CFU) of viable bacteria and fungi that were many orders of magnitude lower than emitted numbers of fluorescent biological particles. Qualitatively, the trend of FBAP >> CFU was expected, as FBAPs are known to include both non-culturable microbes and non-microbial bioaerosols (Bliznakova et al., 2007). Toivola et al. (2002) have shown that airborne concentrations of bacteria and fungi can be two to three orders of magnitude higher when determined by microscopic assessment as compared with culture-based analysis.

Conclusions

Occupant emission rates of coarse fluorescent biological aerosol particles, under seated office-work conditions in a controlled environmental chamber, were evaluated and found to average 0.9 $\pm$ 0.3 million particles/person-h. There was a 5–6$\times$ increase in emission rate associated with walking as compared with sitting, or when upper body movements were enhanced without increasing contact with the floor. Size distributions of emitted particles associated with both walking and sitting conformed well to lognormal distributions with modal diameters of ~3 μm. Based on previous research, the emitted fluorescent biological aerosol particles are expected to contain agglomerated bacteria, fungal spores, human skin flakes, and some non-biological interferents. The quantitative similarity between emissions evaluated in this chamber study and in a previous, observational study conducted in a classroom (Bhangar et al., 2014) supports the inference that the results obtained are robust and may have some general validity. It also supports the utility of emission rate estimates as an important measure for assessing underlying processes across environments, which might themselves have different volumes, occupancy, air exchange rates, HVAC system filtration efficiencies, and contributions from outdoor air.

The apportionment of the occupant-associated source strength to floor vs. non-floor components is of interest from the perspective of evaluating potential health impacts associated with bioaerosol exposure and in designing effective controls. The manipulations conducted in this carpeted chamber indicated that during both walking and sitting, at least 60–70% of emissions originated from the floor. The increase in emission rates during walking vs. sitting was mainly attributable to the intensity of contact with the floor, but may also have had a contribution from the associated increased vigor of upper body movements. Clothing, or possibly the interaction of clothing with human skin, was demonstrated to be a source of FBAPs, and especially of the highly fluorescent fraction. Emitted particles of varying fluorescence intensity followed similar trends in terms of the fractional reduction when the floor was covered, the emissions size mode, and the dependence on activity intensity. The relative contribution of occupants (vs. outdoor air) to indoor concentrations followed an increasing

trend with particle size and particle fluorescence intensity, and was greatest for the highly fluorescent particle fraction.

At present, health impacts linked specifically to bioaerosols emitted by humans are unknown and might conceivably include both positive and negative outcomes. Health effects could depend on source composition profiles of environmental and human bioaerosol reservoirs that feed emissions, including the human body itself, clothing, and indoor dust.

These results set a foundation for evaluating occupant FBAP emission rates for human activities in a range of domestic, workplace, recreational, and transport microenvironments. Future work could usefully assess the magnitude and determinants of interindividual differences, for example whether some people are 'super emitters' and, if so, why. Finally, there is a need to further elucidate the composition of heterogeneous indoor fluorescent particles. That is, to what extent are these biological particles human, bacterial, fungal or other? Efforts are also warranted to develop techniques to identify and characterize non-biological components that may contribute to the fluorescence signal. Insights into the identity of common groups of indoor fluorescent airborne particles will support the use of laser-induced fluorescence as a convenient technique for assessing, with high temporal resolution, sources, and dynamic processes influencing bioaerosols in occupied environments.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Temperature and relative humidity trends during basic and supplementary treatments.

Appendix S2. Preliminary explorations of the influence of relative humidity on FBAP emissions.

Table S1. UV-APS lumped size groups.

Table S2. Air-exchange rate (a) assessments from selected treatments (Tm).

Table S3. The expected mean ($\pm$ standard deviation) indoor proportion of outdoor particles ($IPOP$), evaluated per particle size group as the average indoor/outdoor particle number concentration (based on data from optical particle counters) during zero occupancy basic treatments (normal fan; $N = 6$) and zero occupancy supplementary treatments conducted at reduced fan speeds (reduced fan; $N = 2$).

Table S4. Adjustment factors from OPC side-by-side tests.

Figure S1. (a) The chamber interior (volume ~75 m^3) showing sampling equipment, desks and chairs (with casters) for up to 8 seated occupants, inoperable windows along the south and west walls, the carpeted floor, and floor-level air supply vents. (b) The chamber exterior south wall, showing its location on the building's second story.

Figure S2. Mean concentrations of particles (excluding those with fluorescence intensity = 1) in the chamber air during two representative treatments conducted with the floor exposed: (a) 8 seated occupants (treatment 8), and (b) zero occupants (treatment 7).

Figure S3. A comparison between empirical estimates (means $\pm$ standard deviations, $N = 3$–9) of size-specific deposition loss rate coefficients (k), and values from Thatcher et al. (2002) for conditions that match the airspeed (14 cm/s) measured in the core of the chamber.

Figure S4. UV-APS size calibration curve.

Figure S5. Mean ($\pm$ standard deviation) estimated emission rates of total particles 2–5 μm (ER_{T2}), from basic treatments, when occupants (number = 0, 1, 2, 8) were seated and engaged in normal desk work, with the carpeted floor exposed or with the floor covered with clean plastic sheeting.

Figure S6. Size distributions of weighted mean ($\pm$ standard deviation) occupant emissions of highly fluorescent particle numbers normalized by the mass of carbon dioxide emitted (E_{F20}), shown as a function of particle aerodynamic diameter (d_a) during basic treatments when 1–8 occupants were sitting and engaged in normal desk work, with the carpeted floor (a) exposed or (b) covered ($N = 9$ treatments and 31–33 subjects per floor condition).

Figure S7. Size distributions of weighted mean ($\pm$ standard deviation) normalized occupant emission rates of (a) FBAP numbers (E_F) and (b) highly fluorescent numbers (E_{F20}) during basic ($N = 9$) vs. supplementary treatments ($N = 3$) when 1–8 occupants were sitting and engaged in normal desk work with the carpeted floor exposed.

Figure S8. Size distributions of weighted mean ($\pm$ standard deviation) normalized occupant emission rates of (a) FBAP numbers (E_F) and (b) highly fluorescent numbers (E_{F20}) during basic ($N = 3$) vs. supplementary ($N = 1$) treatments when 2 occupants walked on the exposed carpeted floor.

Figure S9. Time-series of (a) size-integrated (2.5–10 μm) N_{F20} and N_F concentrations, and N_F/N_T concentration ratios, and of (b) size-resolved N_F concentrations, during a treatment with a choreographed series of upper body movements.

Figure S10. Supply-air levels of total particles larger than 2 μm (PN_2), evaluated with optical particle counters, for the non-RH treatments, comparing baseline and treatment periods.

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