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Ambient bioaerosol indices for indoor air quality assessments of flood reclamation

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Abstract

An air quality study was conducted in arid-region residences that were cleaned and reoccupied following a major regional flood (Arkansas River, Colorado, USA). This demonstration study leveraged a suite of aerosol measurements to assess the effects of common flood reclamation practices on indoor air quality. These assays included (i) optical counting (OPC) of airborne particulate matter (0.3–5 μm optical diameter), (ii) composite observations of volatile organic compounds (VOC), (iii) culturing and direct microscopic counts of airborne bacteria and fungi, and (iv) air-exchange rate measurements. As judged by OPC, most of the flood damaged homes surveyed had higher concentrations of airborne particulate matter indoors than outdoors; the same trend was observed for selected VOC. When compared to large literature databases, culturing from air samples collected in houses reclaimed from flood damage had significantly higher airborne microorganism levels than in houses where no flood damage had occurred—in many cases this difference was between two and three orders of magnitude. As determined by direct epifluorescence microscopy, total airborne microorganism concentrations were 3–1000 times higher than those recovered by conventional culturing. In flood damaged homes, biological particles averaged 52% of the total particles measured indoors, and 18% of the total particles measured immediately outdoors. Relative differences between the indoor and outdoor concentrations of airborne particulate matter, microorganisms, and associated VOCs, suggested that flood-impacted building materials were sustaining high aerosol bioburdens and

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contributing to poor indoor air quality more than 3 months after the structures had been reclaimed from flood damage.
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1. Introduction

Poor indoor air quality has been shown to cause adverse health effects. While air quality indices and exposure levels are well defined in terms of certain chemical compounds and particulate matter, they are poorly defined regarding airborne contaminants of microbiological origin. As a generic class of airborne pollutants, particulate matter usually associated with compounds of biological origin is often termed "bioaerosol". This definition includes all airborne microorganisms regardless of viability or ability to be recovered by culture; it comprises whole microorganisms as well as fractions, biopolymers and products from all varieties of living things (ACGIH, 1999). Indoor bioaerosols can originate from outdoor air or from internal sources such as building occupants and their activities, and building materials that host microbiological growth.

Numerous indoor air quality publications report that airborne biological particles range in aerodynamic diameter between 0.01 and 100 μm (ACGIH, 1999). In many indoor environments, airborne bacteria, fungi and their fragments may fall into a respirable size range that can penetrate deep into human lungs (< 10 μm) (Górny et al., 2002; Reponen, Grinshpun, Conwell, Wiest, & Anderson, 2001). Higher respiratory morbidity and allergic complaints have been observed in occupants of mold-colonized structures in several studies (Brunekreef et al., 1989; Dales, Zwanenburg, Burnett, & Franklin, 1991; Platt, Martin, Hunt, & Lewis, 1989; Strachan, 1988; Verhoeff & Burge, 1997; Verhoeff, van Wljnen, & van Brunekreef, 1995). High airborne bacteria concentrations have also been positively correlated to adverse respiratory symptoms (Björnsson et al., 1995). However, bioaerosol concentrations responsible for adverse health effects have not been defined.

Airborne bacteria and fungi can be toxigenic, allergenic and/or infectious. While only complete microorganisms can be infectious, toxic and allergic reactions can be caused by microorganism fragments or byproducts (Burrell, 1991; WHO, 1990). Examples include endotoxin, a compound found in Gram-negative bacteria cell walls (ACGIH, 1999); microbial volatile organic compounds (VOC), products of bacterial and fungal metabolism (ACGIH, 1999; Miller, 1992); β-(1–3)-D-glucans, found in fungal cell walls (ACGIH, 1999); and mycotoxins, products of fungal metabolism (Robbins, Swenson, Nealley, Gots, & Kelman, 2000). Cell and spore fragments can be important sources of allergens and toxins, as their numbers can be several magnitudes higher than cells or spores released from building materials, depending on the species, environmental conditions and wind velocity (Górny et al., 2002).

Fungal and bacterial growth, in and on water-damaged building materials, is a potential health hazard and many recent reports contain evidence to support this observation (Abe & Nagao, 1996; Bardana, 2003; Zureik et al., 2002). The incidence of human disease has been reported to increase markedly following the flooding of residential areas (Marwick, 1997; MMWR, 1993a, b, 1994). While some of these diseases can be traced to waterborne infectious agents and to conventional disease vectors (i.e. mosquitoes), many cannot be linked to specific sources. In this context, there is relatively little information regarding aerosol

exposures within flood damaged residences to suggest an epidemiological link between exposure and adverse health outcomes.

The literature concerning human bioaerosol exposures and associated regulatory limits is tenuous. At present, neither the US Environmental Protection Agency (EPA) nor the National Institution of Occupational Safety and Health (NIOSH) have proposed concentration limits for bioaerosols. One of the earliest guidelines was proposed in 1946 which suggested that no more than 0.1–20 colony forming units $(\mathrm{CFU})/\mathrm{ft}^3$ should grow in 24 h in operating theatres (Topley, 1955). The American Conference of Governmental Industrial Hygienists (ACGIH) reported interim indoor bioaerosol exposure guidelines based on culturable levels of bacteria and fungi, but these guidelines have been repealed since 1999. Those guidelines recommended that less than $100\,\mathrm{CFU/m}^3$ was an acceptable level (ACGIH, 1989). The Health and Welfare department in Canada proposed the following guidelines: (1) $50\,\mathrm{CFU/m}^3$ of one species of fungi warrants immediate investigation; (2) the presence of certain fungal pathogens is unacceptable; (3) $150\,\mathrm{CFU/m}^3$ of mixed species is normal; and (4) up to $500\,\mathrm{CFU/m}^3$ is considered acceptable if the species present are primarily *Cladosporium* (Environment Canada, 1989; WHO, 1990). The European Union also suggested bioaerosol concentration exposure thresholds in terms of CFU, suggesting guidelines for residential and industrial environments (CEC, 1993). More recently, Górny and coworker reviewed European literature databases on residential indoor air quality and proposed the following residential limit values: 5×10^3, $5 \times 10^3\,\mathrm{CFU/m}^3$, and $5\,\mathrm{ng/m}^3$ for airborne fungi, bacteria and bacterial endotoxin, respectively; the presence of pathogenic fungi is considered unacceptable in any concentration (Górny & Dutkiewicz, 2002). In 1994, the New York City Department of Health issued guidelines for assessment and remediation of indoor fungal contamination. This report qualified recommendations in the context of biological indoor air quality problems with the statement "it is not possible to determine "safe" or "unsafe" levels of exposure…" (NYC-DOH, 1994). To determine the presence of significant indoor microbiological sources, these guidelines also recommended comparisons of the species recovered from standard plate counts in addition to comparing the microorganism concentrations recovered from parallel air samples collected indoors and outdoors. These recommendations have become standard for many other organizations (ACGIH, 1999; WHO, 1990), and an extensive review by Rao and Burge lists many organizations and the guidelines they have presented (Rao, Burge, & Chang, 1996).

Most of these guidelines are based on baseline (bio)aerosol concentrations, without taking into account effects on human health (Rao et al., 1996). In addition, most studies have proposed threshold bioaerosol concentrations based on culturing assays (Reponen, Nevalainen, Jantunen, Pellikka, & Kalliokoski, 1992; Reynolds, Streifel, & McJilton, 1990; Robertson, 1997; Yang, Lewis, & Zampiello, 1993). Organizations such as NATO and WHO have concurred that, there is a need to develop more accurate and robust methods for characterizing biological aerosols (Maroni, Axelrad, & Bacaloni, 1995; WHO, 1990). Since many bioaerosol associated diseases are not dependent upon infection to induce adverse health effects, it is important to quantify all microbial cells that are suspended in the air, as well as differentiating between those that are metabolically active, those that are culturable, and those that are non-viable (Hernandez, Miller, Landfear, & Macher, 1999).

A goal of this demonstration study was to compare common and emerging air quality indices observed in a cohort of single-family residences reclaimed after an arid-region flood, to those observed in non-flood impacted homes. A residential demonstration study was performed in Southern Colorado, USA, where, due to heavy rains, the Arkansas River flooded the city of La Junta. Both indoor and outdoor air was sampled several months after the flooding had occurred and after full-scale remediation efforts, when residents had cleaned and returned to their homes. Novel air sampling paradigms and equipment

were used to determine the total airborne bacteria and fungi concentrations within residences after they were reclaimed from flood damage; these were executed in parallel with conventional culturing assays using non-selective media. These concentrations, together with air-exchange rate monitoring, VOC and airborne particulate matter measurements, were used as evidence to determine if the reclamation efforts following flood damage mitigated the potential for significant microorganism enrichments of indoor air (i.e. higher indoor concentrations).

2. Materials and methods

2.1. Microbiological air quality sampling protocol

The following protocols were applied to monitor building air-exchange rates, airborne microorganism concentrations—both total and culturable—and critical environmental factors in the flood-damaged homes.

Air-exchange rates were estimated using tracer gas tests. Thirty minute monitoring of a CO_2 spike (and its subsequent decay) was executed in the main room of the flood-damaged residences. Following CO_2 tracer tests, bioaerosol samples were collected in swirling liquid impingers (3 h) (Willeke, Lin, & Grinshpun, 1998) and conventional N6 Andersen impactors (1 or 2 min) (Andersen, 1958), while total airborne particle concentrations in the size range between 0.3 and 5 μm optical diameter (OD), were concurrently monitored for up to 4 h. Temperature and relative humidity were recorded hourly during the sampling campaigns.

2.2. Residence selection

Indoor and outdoor air samples were collected and characterized in eight single story flood-damaged houses and one non-flooded house. Building selection was based on similarity in extent of flood damage, the structure (single level), age and construction materials, as well as remediation status (complete). Cleaning was considered complete when wetted carpets had been replaced, soaked dry walls and subfloors had been patched or replaced, non-structural surfaces had been washed with bleach, and forced-air dryers had been applied. Air sampling was executed between 2 and 3 months following their cleaning and reoccupation. This coincided with the summer season, when outdoor bioaerosol concentrations have been implicated as the major source of indoor bioaerosol concentrations in residential buildings (Nevalainen, Pasanen, Reponen, Kalliokoski, & Jantunen, 1991). Passive ventilation (open windows and doors) was the main method used to ventilate these residences when occupied during the summer months.

Residents carried out their normal activities up to a couple of hours before air sampling commenced. Because of the short-term effects of everyday activities on indoor bioaerosol concentrations (Lehtonen, Reponen, & Nevalainen, 1993), there were no human or animal activities in the residences during the sampling campaigns. Special care was taken not to disturb the residences' interiors; this practice was meant to minimize particle reaerosolization and provide for sampling normalization among the residences sampled.

2.3. Environmental monitoring

Temperature and humidity probes (Fisher Scientific, Fullerton, CA) monitored relative humidity and temperature hourly, both indoors and outdoors, during all sampling periods. To minimize temporal

variations, tracer gas studies were executed, and indoor and outdoor air was sampled at the same times, between 9 am and 2 pm, in every residence. Wind speed data and general weather conditions were obtained from a local meteorological station (La Junta Municipal Airport, La Junta, CO).

2.4. Air-exchange rates

Tracer gas tests were used to estimate air-exchange rates of the residences under the conditions monitored; these CO_2 tests were modified from a widely accepted decay method (Kronvall, 1981; Winberry et al., 1993). The protocol for the decay test was as follows: CO_2 gas was injected in the residences, and allowed to mix and accumulate to a level of 5000 parts-per-million (ppm). Once 5000 ppm was reached, CO_2 injection was ceased and the CO_2 concentration was recorded every minute until the gas had reached background levels (typically 800 ppm indoors). Carbon dioxide was used as a tracer because it is a non-reactive gas that is easy to monitor and does not pose a health threat at the concentrations used. CO_2 was measured using a Langan CO_2 probe fitted with a microprocessor for continuous data acquisition (Langan Products, Inc., San Francisco, CA).

Indoor air mixing was facilitated by small household 120 V box fan (33 cm diameter) placed in the rooms sampled. To reduce the potential for spore release from building materials (Górny, Reponen, Grinshpun, & Willeke, 2001; Pasanen, Pasanen, Jantunen, & Kallikoski, 1991), mixing fans were placed in a manner that did not direct airflow towards the walls. Fans were operated according to the following protocol: ON during tracer gas injection and bioaerosol sampling, and OFF during CO_2 monitoring.

2.5. Microbiologically associated volatile organic compounds (MVOC)

Air samples for selected VOC analyses were drawn into a glass tube containing activated carbon media (Air Quality Sciences, Marietta, GA) using a pump (model 224-PCXR8, SKC Inc., Eighty Four, PA) for 4 h at a flow rate of 0.2 L/min, collecting 48 l of air. Tubes were placed approximately 2 m above the ground, hanging vertically from a rack. Care was taken to place tubes away from walls or close to other potential VOC sources. At the end of the sampling period, tubes were shipped overnight on ice and analyzed with a gas chromatograph/mass spectrometer using widely accepted methods (AQS, 1997). Based on the laboratory equipment sensitivity and volume collected, detection limits for the compounds reported were 10 ng/m^3.

2.6. Bioaerosol collection and analyses

2.6.1. Swirling liquid impingers: BioSamplers

Bioaerosol samples were collected using swirling liquid impingers according to accepted methods (Lin et al., 1999, 2000; Willeke et al., 1998) and manufacturer's specifications (BioSampler, SKC Inc., Eighty Four, PA). The efficiency of the BioSampler filled with 20 ml of water is 79% for 0.3 μm particles, 89% for 0.5 μm particles, 96% for 1 μm particles and 100% for 2 μm particles (Willeke et al., 1998). Particle-free, autoclaved 0.01 M phosphate-buffer saline (PBS) containing 0.01% Tween 80 (SIGMA, St. Louis, MO) was used as the collection medium in all impingers. For bioaerosol sampling, three BioSamplers were placed in clusters at least 1 m above the ground, indoors and outdoors. Outdoor samples were located at least 1 m above the ground, several meters away from open doors and windows to minimize the influence from indoor sources. If samplers had to be placed closer to doors, these were kept shut during

the experiments and alternate routes of entry were used to check the indoor samplers. The BioSampler inlets were oriented such that their directions defined the points of an equilateral triangle, which provided multidirectional collection and reduced any near-field sampling effects the impingers may have had on each other. All impingers were connected to a rotary vane-type vacuum pump (model 1023-101Q-G608X, Gast Inc., Benton Harbor, MI) and collected air at a flow rate of 12.5 L/min (SD = 0.7 L/ min). The vacuum pumps were operated for 5 min prior sampling to assure a constant vacuum source. Flow rates were monitored by three 50 L/min capacity flow meters (Gilmont® Instruments, Barrington, IL) and calibrated with a primary standard airflow bubble meter (Gilibrator, Gilian Instrument Corp., Clearwater, FL).

BioSamplers were operated for a minimum of three consecutive hours during which time they collected 2250 L of air. During extended BioSampler operations, the reservoir liquid evaporates, which can lead to collection efficiency reductions from re-aerosolization and particle bouncing (Lin et al., 1999; Willeke et al., 1998; Grinshpun et al., 1997; Lin et al., 1999). To keep collection efficiency constant, a sterile phosphate saline buffer solution was periodically added to maintain the impingers' reservoir volumes at the manufacturer's recommended level of 20 ml. Buffer was prepared and autoclaved in the laboratory, and, as a precaution, was filter sterilized on-site using a Nalgene vacuum bottle fitted with a 0.2 μm pore filter just prior to using. Approximately every 30 min the pumps were turned off and any evaporated capture buffer was quickly replaced by injecting sterile buffer down the impingers' neck. For this study, which was executed in an arid region with low humidity, it was necessary to replace approximately 4 mL (±1 mL) of buffer every half-hour to keep the manufacturer's recommended liquid levels within the impingers' reservoirs. Before sampling, impingers were washed with deionized water and 70% ethanol and autoclaved for 15 min at 121 °C. Immediately after collection, samplers were stored on ice to minimize microorganism growth, and shipped to the University of Colorado environmental microbiology laboratory (within 4 h) where their contents were aseptically diluted for direct microscopy, and transfer onto agar plates.

2.6.2. Microorganism enumeration: culturability assays via liquid capture

A modification of a standard plate count method (Gerhardt, Murray, Wood, & Krieg, 1994) was used to enumerate culturable bacteria and fungi retained in the impinger's liquid. Within 4 h after collection, liquid samples from impingers were cultured on plates inoculate by a spiral dispenser (Spiral Biotech, Inc., Bethesda, MD) according to the manufacturer's recommendations. At least three replicates of each sample were cultured. A comparison of culturable counts determined with the spiral plater, and those determined by standard spread plate methods, showed no significant differences between the recovery of these methods (based on an independent t-test, $\alpha = 0.05$), and that the spiral plater method variability was lower than that of the spread plate method (coefficient of variance (CV) was 5% lower for the spiral plating method, $n = 10$).

For culturing assays, agar plates were prepared up to a week in advance and stored under aseptic conditions. Culture plates were refrigerated at 10 °C prior to use, and care was taken to avoid the drying effects of long exposures to room temperature or direct sunlight. Bacteria were cultured on tryptic soy agar (TSA) (Difco Laboratories, Detroit, MI) including 0.5% cycloheximide (SIGMA, St. Louis, MO) to prevent fungal growth (Schillinger, Vu, & Bellin, 1999). Fungi were cultured on malt extract agar (2% MEA) (Difco Laboratories, Detroit, MI), which is recommended by the American Conference of Governmental Industrial Hygienists (ACGIH) as a non-selective fungal agar (ACGIH, 1999) including 0.05% chloramphenicol (SIGMA, St. Louis, MO) to inhibit bacterial growth (Schillinger et al., 1999).

This broad-spectrum fungal medium has been recommended for determination of building associated fungi (Samson et al., 1994). Once inoculated, bacterial plates were incubated at 37 °C for 14 days, and CFUs counted every 3 days. Fungal media plates were incubated at 25 °C for 14 days and CFUs counted every 3 days.

2.6.3. Microorganism enumeration of impinger reservoir contents: microscopy assays (total microorganism counts)

Epifluorescence microscopic counting was used to enumerate the total numbers of bacteria and fungi (culturable, and non-culturable) captured in impinger samples. For microscopy, cells were stained with Acridine Orange (AO) (Fisher Scientific, Springfield, NJ), a fluorescent stain that non-selectively binds to nucleic acids (Hobbie, Daley, & Jasper, 1977). Samples for total cell counts were stained at a final concentration of 0.001% AO, incubated for 1 min at room temperature, and filtered through a 25 mm diameter black polycarbonate filter with a pore size of 0.2 μm (Poretics, Inc., Livermore, CA). All direct counts were reported based on counts from the average of 10 microscopic fields. Mounted filters were examined under 1000× magnification using a Nikon Eclipse E400 epifluorescence microscope fitted with a mercury lamp and polarizing filters (HBO-100 W mercury lamp; F/TXRD X excitation filter; F/TXRD M emission filter; F/TXRD BS beamsplitter (ChromaTechnology Corp., Brattleboro, VT)). A 24-bit color digital camera (Spot Camera, Diagnostic Instruments, Sterling Heights, MI) captured fluorescent micrographs, which were then viewed and archived using Adobe Photoshop 5.0 software (Adobe Systems, San Jose, CA).

2.6.4. Microorganism enumeration: culturability assays via solid agar capture in Andersen impactors

A one-stage N6 Andersen impactor (Graseby-Andersen Instruments, Smyrna, GA) was used to compare impaction recovery of airborne bacteria and fungi to that obtained using BioSamplers. This stage collects particles with a 50% cut-off aerodynamic diameter (d_{50}) of 0.65 μm. Impactors were connected to a vacuum pump (model 10709, Andersen Samplers Inc., Atlanta, GA), which collected air at 28.3 L/min. Impactor pumps were calibrated using a bubble meter (Gilibrator, Gilian Instrument Corp., Clearwater, FL). Either 28.3 or 56.6 L of air were collected for each sample (1 or 2 min sample time). The impactor equipment was washed and sterilized with 70% ethanol prior to sampling, and the impactor was operated for 30 s with a sterile, HEPA filtered air to purge any microorganisms trapped from previous handling. Blanks were included to verify sterility. Impactors were placed 1.5 m above the floor, more than 3 m from the BioSamplers. One indoor and one outdoor impactor sample was collected in each house.

Agar plates loaded into the impactor were prepared according to manufacturer's recommendations, and media plates were incubated and counted as previously outlined. Colony counts were adjusted with a positive-hole correction factor to account for the possibility of collecting multiple particles through single holes on the Andersen sampler stages (Macher, 1989).

2.6.5. Total particle counts

An optical particle counter (OPC) model 237B (Met One, Pacific Scientific Company, Chandler, AZ) was used to count as a function of size total (biological and non-biological) particles collected both indoors and outdoors. The particle counter was connected to a timer and solenoid valve that switched between indoor and outdoor sampling every minute. Sampling volume was 1.4 L, collected for 30 s at a flowrate of 2.8 L/min. Particle concentrations were recorded in the following size ranges on the basis of

optical diameter: 0.3–0.5, 0.5–0.7, 0.7–1, 1–2 and 2–5 μm. One hundred samples were collected at each residence, 50 indoors and 50 outdoors, over a time frame of 100 min.

3. Results

3.1. Environmental monitoring

During the sampling periods (between 9 am and 2 pm, 5 h for a typical residence), temperatures indoors and outdoors increased, while relative humidity decreased. In the flood-damaged houses, relative humidity indoors varied between 43 and 88%, and outdoors between 31 and 85%. Temperatures varied between 20 and 28 °C indoors, and between 17 and 35 °C outdoors. Within a single observation, the maximum relative humidity variation was ±7% indoors and ±19% outdoors; the maximum temperature variation was ±2 °C indoors and ±3.2 °C outdoors. Wind speed on the days of the monitoring varied between 8.5 and 16 km/h. Based on the CO_2 decay experiments, air-exchange rates in the houses varied between 0.8 and 3.5 air changes per hour (ACH, 1/h).

3.2. Microbiologically associated volatile organic compounds

Selected VOCs were monitored as indicators of fungal metabolism (ACGIH, 1999; AQS, 1997; Miller, 1992; Pasanen, Lappalainen, & Pasanen, 1996). VOC of possible microbial origin (MVOC) were detected in over half of the flooded houses tested. Three alcohols and one ketone were detected in significant concentrations, varying between 70 and 2710 ng/m^3. The most common VOC found was 3-methyl-1-butanol, which has been associated with fungal growth on building materials (AQS, 1997). Other common MVOC found were 2-octen-1-ol, 2-heptanone, and 1-octen-3-ol. Fig. 1 summarizes the type and quantity of MVOC observed in all houses surveyed.

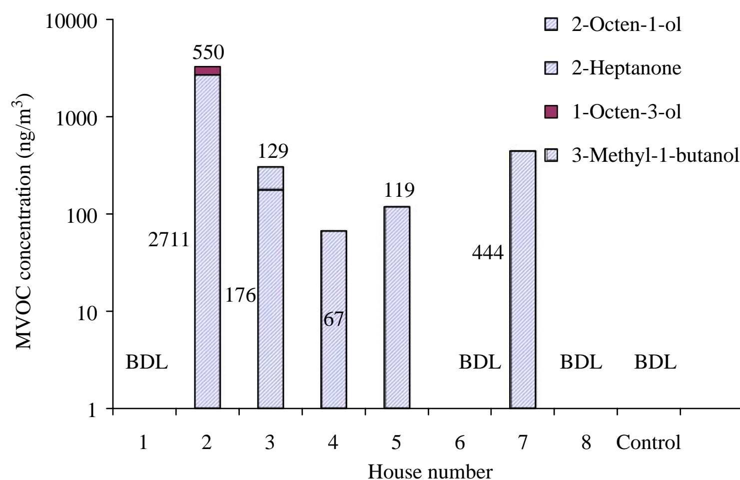


Fig. 1. Type and quantity of microbial volatile organic compound (MVOC) extracted from 48 L of indoor air in flood-damaged and control residences. All outdoor samples collected were below the VOC detection limit. BDL = below detection limit (10 ng/m^3).

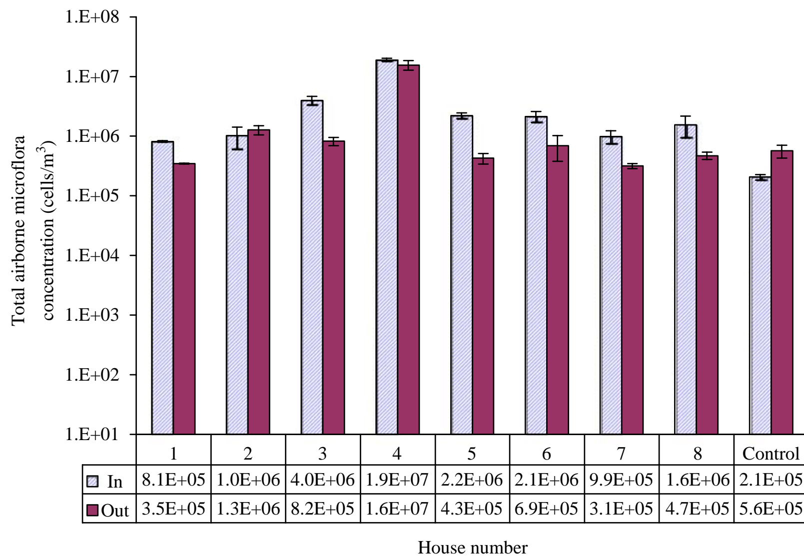


Fig. 2. Average total airborne bacteria and fungi concentrations recovered from SKC swirling liquid impingers in flood-damaged residences, as determined by direct microscopy. Error bars represent one standard deviation, $n = 3$.

3.3. BioSamplers—total airborne microorganism recovery

In all flood-damaged houses, total indoor airborne microorganism concentrations ranged between 8.1×10^5 and 1.9×10^7 cells/m^3, and outdoor concentrations ranged between 3.1×10^5 and 1.6×10^7 cells/m^3. Fig. 2 summarizes total airborne microorganism level, as defined by the sum of all bacteria, fungi and spores observed in and near the houses. As judged by *t*-test at a 95% probability level ($\alpha = 0.05$), seven of eight flooded houses had indoor microorganism concentrations significantly higher than their corresponding immediate outdoor concentrations; one flooded house (house #2) did not show a statistically significant difference between indoor and outdoor total microorganism concentrations, and the local control house had indoor concentrations significantly lower than that measured immediately outdoors. There was a broad diversity of microscopic cellular morphology observed in all the samples collected, and no general trends in morphology were observed. Propagule sizes ranged from less than 1 μm to over 10 μm in diameter. Fig. 3 is an epifluorescence microscope photograph of AO-stained microorganisms typical of those recovered from the air inside flood-damaged houses.

3.4. SKC liquid impingers—culturable recovery

3.4.1. Bacteria

Mesophilic bacteria were recovered from the SKC liquid impingers on non-selective media (TSA). Seven of the eight flooded houses had higher averages of airborne culturable bacteria concentrations indoors than outdoors (Fig. 4), although only four were statistically different as judged by means and analyses of variance (*t*-test, $\alpha = 0.05$).

Averages of culturable airborne bacteria recovered from indoor air of flood-damaged homes ranged between 3.9×10^2 and 3.9×10^5 CFU/m^3, while corresponding outdoor concentrations ranged between

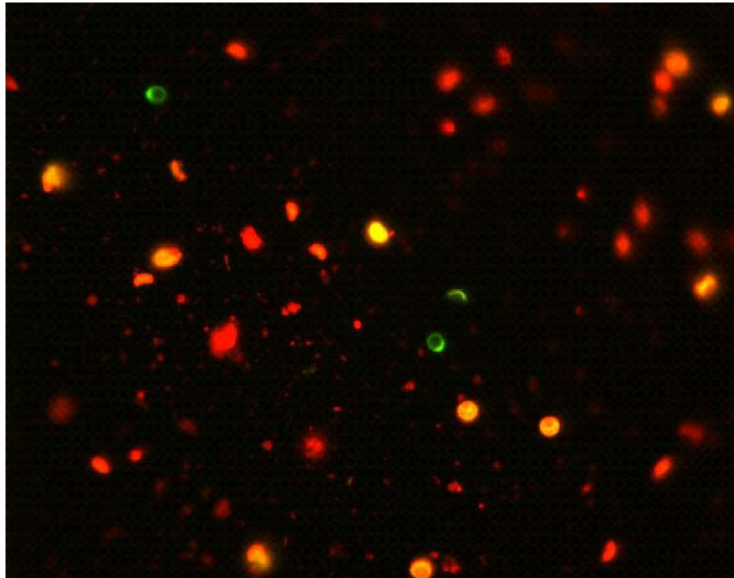
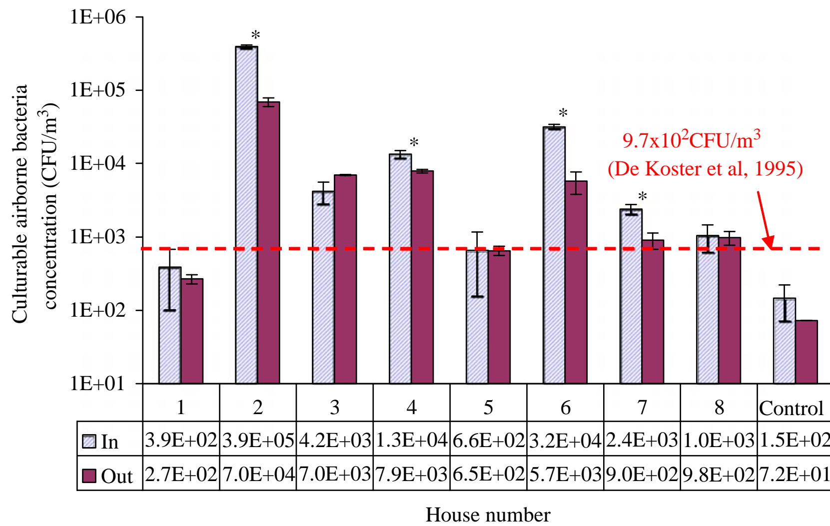


Fig. 3. Epifluorescence microscope photograph of AO-stained bacteria, fungi, and spores collected from the indoor air of a flood-damaged home (1000×).



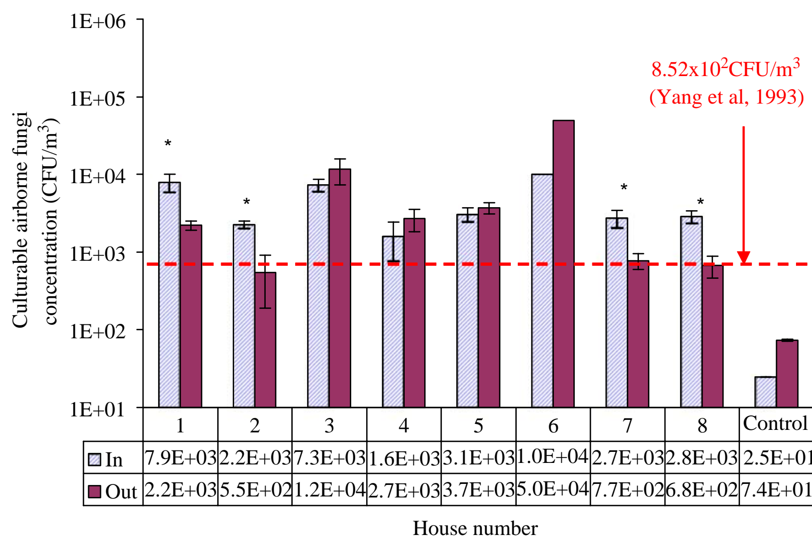
	1	2	3	4	5	6	7	8	Control
In	3.9E+02	3.9E+05	4.2E+03	1.3E+04	6.6E+02	3.2E+04	2.4E+03	1.0E+03	1.5E+02
Out	2.7E+02	7.0E+04	7.0E+03	7.9E+03	6.5E+02	5.7E+03	9.0E+02	9.8E+02	7.2E+01

Fig. 4. Average airborne concentrations of culturable bacteria recovered from BioSamplers. Error bars represent one standard deviation, $n = 3$. Asterisks denote houses where concentrations were statistically different indoors and outdoors. A line represents the average value of culturable bacteria from a survey of non-flood-damaged US homes, $n = 41$ (DeKoster & Thorne, 1995).

2.7×10^2 and 7.0×10^4 CFU/m^3. The ratios of airborne bacterial concentrations recovered indoors and outdoors varied between 3.5 and 8.8. In a non-flooded residence in the local vicinity, average indoor concentrations were less than 33% of the immediate outdoor concentrations, a ratio which was in agreement with many previous observations (Nevalainen et al., 1991; Samson, 1985; Solomon, 1975; Verhoeff, Brunekreef, Fischer, van Reenen-Hoekstra, & Samson, 1992).

3.4.2. Fungi

Impinger-captured aerosol samples were cultured on malt extract agar to maximize the recovery of fungi and their spores. Culturable concentrations of airborne fungi were generally higher indoors than outdoors, and the dominant types of fungal genera cultured from indoor air samples were different from those cultured from outdoor samples. On this non-selective fungal media, four of eight houses had significantly higher culturable concentrations of fungi indoors than outdoors (t-test, $\alpha = 0.05$) (Fig. 5). Average concentrations of culturable fungi from air samples inside flooded houses varied between 1.6×10^3 and 1.0×10^4 CFU/m^3, and immediately outside flooded houses between 5.5×10^2 and 5.0×10^4 CFU/m^3. *Trichoderma* spp. was the colony-forming phenotype most often recovered from indoor air samples, but was not recovered in numerically significant CFUs from any outdoor air samples. *Penicillium* spp. was the colony-forming phenotype most often recovered from outdoor air samples, but was not recovered in numerically significant CFUs from indoor air samples. *Trichoderma* grows optimally in environments with high water activity (Kredics et al., 2004) while *Penicillium* species can grow at a wide range of water activity (Andersen & Frisvad, 2002; Gock, Hocking, Pitt, & Poulos, 2003; Plaza, Usall, Teixidó, & Viñas, 2003). These results indicated that even though the houses had undergone remediation efforts, some building materials were not dry and were promoting the growth of some fungi with an affinity to high water content environments.



	1	2	3	4	5	6	7	8	Control
In	7.9E+03	2.2E+03	7.3E+03	1.6E+03	3.1E+03	1.0E+04	2.7E+03	2.8E+03	2.5E+01
Out	2.2E+03	5.5E+02	1.2E+04	2.7E+03	3.7E+03	5.0E+04	7.7E+02	6.8E+02	7.4E+01

Fig. 5. Average airborne concentrations of culturable fungi recovered from BioSamplers. Error bars represent one standard deviation, $n = 3$. Asterisks denote houses where concentrations were statistically different indoors and outdoors. A line represents the average value of culturable fungi in non-flood-damaged US buildings, $n = 2000$ (Yang et al., 1993).

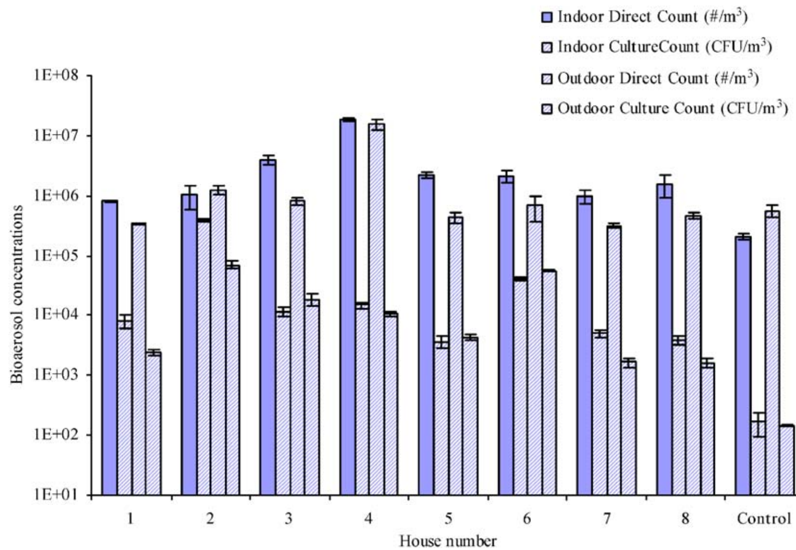


Fig. 6. Direct microscopic counts and culturable CFUs obtained from indoor and outdoor air samples collected with BioSamplers. Error bars represent one standard deviation.

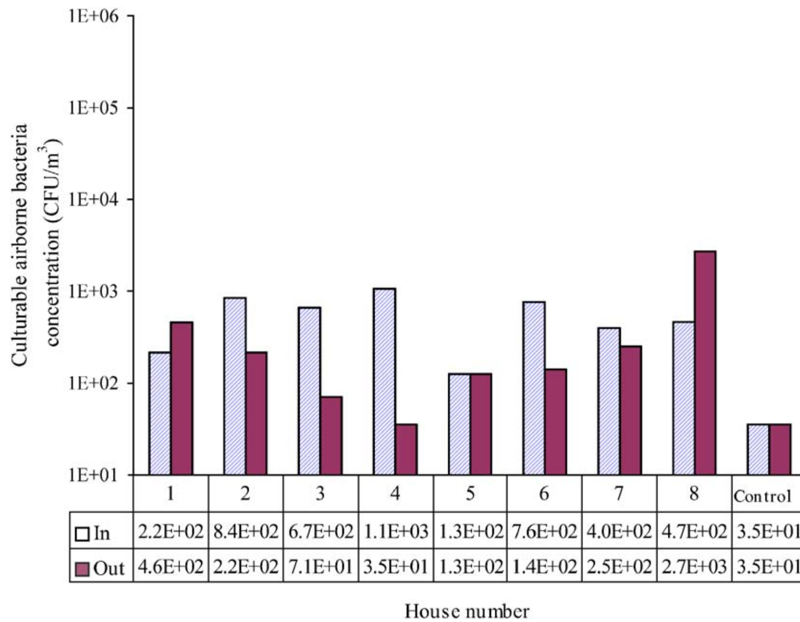
3.5. Comparing direct microscopic counts and culturing recovery from BioSamplers

To compare the recovery of direct microscopic counts and CFUs, both obtained from liquid impinger samples, bacteria and fungi cultured on non-selective media were summed and compared to direct microscopic counts (Fig. 6). Significant differences between concentrations were determined with *t*-tests ($\alpha = 0.05$). Based on direct microscopic counts, seven of eight houses had significantly higher indoor microorganism concentrations compared to outdoors (houses #1, 3, 4–8), a trend which was opposite of that observed in the local control house as well as that reported in larger culture-based surveys (ACGIH, 1999). Based on summed culture counts (i.e. bacteria+fungi), only five houses had significantly higher indoor microorganism concentrations than out (houses # 1, 2, 4, 7, and 8), and no significant difference was observed in the local control house. Indoors, direct counts were 3 to over 1000 times higher than culturable counts obtained from the same indoor air samples while outdoors direct counts were 12 to over 1000 times higher than culturable counts. Although direct microscopy counts were often orders of magnitude higher than culturable counts, these concentrations were poorly correlated (R^2 values 0.004 indoors and 0.02 outdoors). This indicates that culturable counts likely underestimate total microorganism bioburden and cannot predict the magnitude of airborne biological contamination.

3.6. Andersen impactor—culturable recovery

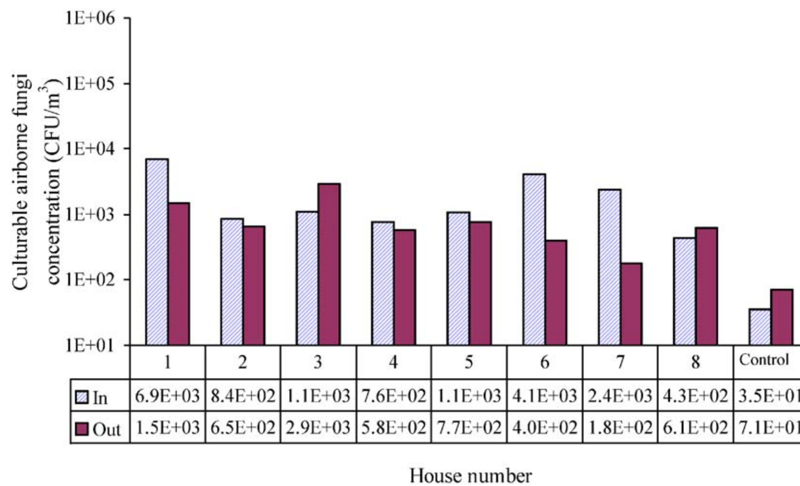
3.6.1. Bacteria

Bacterial colonies cultured on impactor-mounted TSA plates ranged between 1.2×10^2 and 1.1×10^3 CFU/m^3 indoors, and between 3.6×10^1 and 2.7×10^3 CFU/m^3 outdoors (Fig. 7). Inside five out of the eight flooded houses sampled, counts of culturable airborne bacteria were significantly higher (*t*-test, $\alpha = 0.05$) than those measured immediately outdoors, varying between a factor of 1.6 and 30.



	1	2	3	4	5	6	7	8	Control
In	2.2E+02	8.4E+02	6.7E+02	1.1E+03	1.3E+02	7.6E+02	4.0E+02	4.7E+02	3.5E+01
Out	4.6E+02	2.2E+02	7.1E+01	3.5E+01	1.3E+02	1.4E+02	2.5E+02	2.7E+03	3.5E+01

Fig. 7. Estimated airborne concentration of culturable bacteria recovered from one-stage N6 Andersen impactor ($d_{50} = 0.65\,\mu$m).

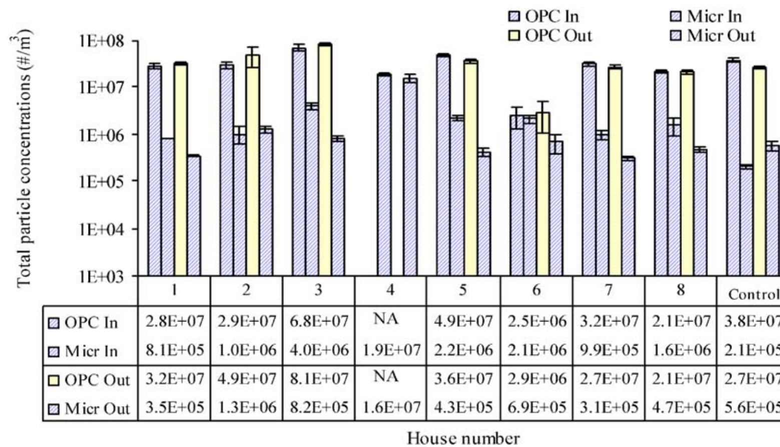


	1	2	3	4	5	6	7	8	Control
In	6.9E+03	8.4E+02	1.1E+03	7.6E+02	1.1E+03	4.1E+03	2.4E+03	4.3E+02	3.5E+01
Out	1.5E+03	6.5E+02	2.9E+03	5.8E+02	7.7E+02	4.0E+02	1.8E+02	6.1E+02	7.1E+01

Fig. 8. Estimated airborne concentration of culturable mesophilic fungi recovered from one-stage N6 Andersen impactor ($d_{50} = 0.65\,\mu$m).

3.6.2. Fungi

Concentrations of airborne fungi cultured on MEA plates varied between 4.3×10^2 and 6.9×10^3 CFU/m^{3}indoors, and immediately outdoors they ranged between 1.8×10^2 and 2.9×10^3 CFU/m^3 (Fig. 8). Inside four out of the eight flooded houses sampled, counts of culturable airborne bacteria were



	1	2	3	4	5	6	7	8	Control
OPC In	2.8E+07	2.9E+07	6.8E+07	NA	4.9E+07	2.5E+06	3.2E+07	2.1E+07	3.8E+07
Micr In	8.1E+05	1.0E+06	4.0E+06	1.9E+07	2.2E+06	2.1E+06	9.9E+05	1.6E+06	2.1E+05
OPC Out	3.2E+07	4.9E+07	8.1E+07	NA	3.6E+07	2.9E+06	2.7E+07	2.1E+07	2.7E+07
Micr Out	3.5E+05	1.3E+06	8.2E+05	1.6E+07	4.3E+05	6.9E+05	3.1E+05	4.7E+05	5.6E+05

Fig. 9. Comparison of OPC-measured particle concentrations (OPC) with epifluorescence counts of microbiological particles (Micr), inside and immediately outside flood-damaged and non-flood-damaged houses. Error bars represent 1 standard deviation.

significantly higher (t-test, $\alpha = 0.05$) than those measured immediately outdoors, varying between a factor 1.3 and 13.5.

3.7. Total particle number concentrations

Between 70 and 94% of indoor particles, and 62–92% of outdoor particles were measured in the first OPC channel (particle optical diameter between 0.3 and 0.5 μm). Between 4 and 15% of indoor particles, and 5–16% of outdoor particles were measured in the second OPC channel (particle optical diameter between 0.5 and 0.7 μm). Less than 1% of particles observed by the OPC were between 2 and 5 μm. Total airborne particle concentrations indoors varied between 2.5×10^6 and 6.8×10^7 particles/m^3 and outdoors between 2.9×10^6 and 8.1×10^7 particles/m^3 (Fig. 9). Total particle concentration information for house #4 was lost due to equipment malfunction. Indoor and outdoor total particle concentrations were not significantly different in five of the eight flooded houses.

While in all cases, the total particle counts (OPC) were higher than those obtained by direct microscopic counting in corresponding size ranges, the biological contribution to the total particle numbers was markedly different indoors and out. On average, biological particles accounted for 52% of the total particles indoors and 18% of the total particles immediately outdoors, of the flooded houses observed. In the house that did not experience flooding, the trend was reversed, and airborne microbiological particles, respectively, accounted for 3% and 20% of indoor and outdoor airborne total particle numbers. The particle counts from the first channel of the OPC were excluded from this analysis, because whole bacteria and fungi cells typically have diameters greater than 0.5 μm. In order to compare total airborne particle numbers with microbiological particle numbers determined by microscopy, OPC readings from channels counting particles with optical diameters between 0.5 and 5 μm were summed. Particle number concentrations determined by OPC had weak correlation with microorganism numbers collected by the SKC biosamplers ($R^2 = 0.04$ for indoor, $R^2 = 0.14$ for outdoor). Better correlations resulted when OPC

readings for particles with optical diameters $< 0.5\,\mu m$ were included in the comparison: $R^2 = 0.24$ for flooded indoor environments, and $R^2 = 0.18$ for those immediately outdoors.

3.8. *Bioaerosol sampling variability and observations of "control" residence*

A one-way analysis of variance ($\alpha = 0.05$) applied to microorganism concentrations, both total and culturable, from three impinger sample points indoors showed that the three samples collected at different locations were statistically indistinguishable. The same test applied to the two outdoor sample points yielded the same results.

Total microorganism concentrations in flood-damaged houses were between 1 and 5 times higher indoors than immediately outdoors, indicating an indoor microbial source. For a single non-flooded house included in this survey, the opposite condition existed: the indoor concentration was 33% of the outdoor concentration, which is a value consistent with those commonly observed in non-flood impacted residences and buildings (DeKoster & Thorne, 1995; Lehtonen et al., 1993; Rautiala, Reponen, Nevalainen, Husman, & Kalliokoski, 1998; Robertson, 1997; Yang et al., 1993).

4. Discussion

4.1. *Environmental monitoring*

Air-exchange rates were monitored concurrently with selected bioaerosols and other airborne particulate matter. The air-exchange rates in the monitored residences varied between 0.8 and 3.5 1/h. This range extends significantly higher than other residential air-exchange rates recorded for the same geographic area and season (Murray & Burmaster, 1995), and may be attributed, at least in part, to the local wind speeds (8.5–16 km/h (daily avg.)). Indoor CO_2 concentrations varied between 300 and 420 ppm in all the houses observed. These relatively low indoor CO_2 concentrations indicated that airborne pollutants are likely not being accumulated because of lack of ventilation (DeKoster & Thorne, 1995).

4.2. *Microbial associated volatile organic compounds*

The most often observed VOC was 3-methyl-1-butanol, which is a VOC commonly associated with fungal growth. Other VOC measured in flood-damaged homes included: 2-octen-1-ol, 2-heptanone and 1-octen-3-ol. Based on recent literature (ACGIH, 1999; Miller, 1992; Miller, Ross, & Moheb, 1998; Pasanen et al., 1996) the types of VOC measured in the flood-damaged homes were consistent with an indoor enrichment of microorganisms with respect to outdoor sources. Given the relatively high air-exchange rates measured, the levels of specific microbial VOCs were significant in magnitude, and indicate the presence of active generation sources. While some MVOC have been implicated as good indicators of indoor fungal growth, they cannot be used to quantify fungi, either airborne or surface associated, or be related to specific fungi. Nonetheless, MVOC can serve as a signature to the indoor enrichment of environmental fungi given that artificial sources are considered, and that a baseline indoor/outdoor ratio is established. As outlined in review and compared to previous studies (AQS, 1997; Brown, Abramson, & Gray, 1994; Lewis & Zweidinger, 1992), the levels and type of VOC observed in this study were indicative of indoor microorganism enrichment. In the house with the highest MVOC measurement

(House 2) however, a person had smoked prior to air sampling. Tobacco smoke contains hundreds of VOC and some of them may have the same chemical signature as many MVOCs (Molhave, 1992). Five of the eight flooded houses had significant MVOC levels, and these observations corresponded to the houses with the highest averages of culturable airborne bacteria. The house with the highest MVOC concentrations also had the highest culturable microorganism counts recovered from the BioSamplers.

4.3. Comparing culturable airborne microorganism recovery in Andersen impactors and BioSamplers

4.3.1. Bacteria

In five out of eight flood-damaged houses, indoor culturable bacteria concentrations were higher than outdoors (*t*-test; $\alpha = 0.05$). Bacterial CFUs recovered on TSA plates in Andersen impactors agreed with the general trends observed from culturing microorganisms retained in BioSamplers: concentrations of culturable airborne microorganisms recovered from the samples collected indoors were consistently higher than those recovered from outdoors. However, bacteria concentrations recovered with the BioSamplers were significantly higher than those recovered with the Andersen in eight of 9 houses tested, in some cases the differences were greater than two orders of magnitude. A possible reason for these differential recoveries is that the sampling stress incurred by airborne microorganisms recovered by liquid impingers is less than those recovered by impactors. This differential sampling stress response has been previously reported in controlled bioaerosol chamber studies (Stewart et al., 1995).

4.3.2. Fungi

Indoor concentrations of airborne fungi cultured on non-selective medium were significantly higher indoors in six of eight flood-damaged residences.

CFUs from Andersen impactors agreed with general trends observed from culturing fungi from samples retained in the BioSampler: concentrations of culturable airborne fungi recovered from the samples collected indoors were consistently higher than those from outdoor samples. Comparing the concentrations of culturable fungi recovered from Andersen impactors and those retained in BioSamplers, the CFUs recovered by the impactor were between 10^2 and 10^3 times less than those recovered by the impinger. Possible reasons for these differences include: (1) the impinger sampling time (hours), was much longer than the impactor (minutes); (2) retention differences intrinsic to the equipment—impactors are subject to particle bounce where (swirling) liquid capture minimizes particle reentrainment; (3) particle stress—impactor particles are subject to impaction and desiccation, where particles in the impinger were collected in swirling liquid and not subject to impaction and dessication; (4) differences in particle-size collection: the impactor collects particles with a 50% cut-off at an aerodynamic diameter of 0.65 mm, whereas the BioSampler has an efficiency of 79% for 0.3 μm particles, 89% for 0.5 μm particles, 96% for 1 μm particles and 100% for 2 μm particles.

4.4. Epifluorescence microscopic counting vs. traditional culturability assays

In most bioaerosol studies, the detection and quantification of metabolically active microorganisms has been primarily based on plate count assays in which sample collection methods as well as microorganism nutritional requirements and culturability potential bias the results (Hernandez et al., 1999). For this study both culturing and microscopy techniques were used because of the synergy of information that can be obtained from these different counting techniques. Fig. 6 suggests that traditional culturing techniques

are inadequate to represent the true quantities of airborne microorganisms in these indoor environments. Direct counts were 3 to over 1000 times higher than CFUs obtained from indoor airborne particulate matter that was captured in the impingers' reservoirs. Outdoors, direct counts were 12 to over 1000 times higher than CFUs from the same sample aliquots. Even though a high fraction of bacteria and fungal suspended in aerosols may not be viable or culturable, they may retain some potential to induce hypersensitivity and inflammatory disease since such responses have no dependence on microorganism culturability to induce adverse health effects (Flannigan et al., 1991). The investigation adds to a small but growing body of bioaerosol literature suggesting that are formidable differences in culturable and total airborne microorganism numbers present in indoor and outdoor environments. These results suggest that direct counts of airborne microorganisms should be included as a critical component of common exposure assessment paradigms.

Only one home was used in a local control capacity in this study because the literature contains a large bioaerosol monitoring database of non-flood-damaged single and multiple family residences (ACGIH, 1999). These studies report that, under normal residential conditions (no water damage), indoor bioaerosol concentrations are significantly lower than outdoor bioaerosol concentrations during summer season (DeKoster & Thorne, 1995; Lehtonen et al., 1993; Rautiala et al., 1998; Robertson, 1997; Yang et al., 1993). Some of these studies compile observations from over 2000 houses, most of which are based on impactor capture, and independent, broad-spectrum culture of bacteria and fungi as described herein. The results obtained from the "non-flood impacted house" in this study agreed with the large literature database: indoor culturable bioaerosol concentrations were, on average, 33% of outdoor concentrations. With regard to culture-based assays of air samples, this observation is widely reported in the literature not only as the more common residential condition, but the favorable one (ACGIH, 1999).

4.5. Comparison of total particle counts with direct microscopy count

In all cases, the total particle counts (OPC) were higher than those obtained by direct microscopic counting in corresponding size ranges. Differences in microbiological contributions to total airborne particle numbers (both in and outdoors) indicate that this ratio may be a useful index for assessing relative aerosol (bio)burdens in residences flood damaged. As judged by particle numbers, results suggest that indoor sources contributed a significant portion of microorganisms to the airborne particulate matter loads in the flood damaged houses observed. However, weak correlations between direct microscopic counts and total particle counts suggest that optical particle counting will not likely be useful for estimating airborne microorganism concentrations in these environments until a larger data base is compiled.

5. Conclusions

In spite of remediation efforts, indoor bioaerosol concentrations observed in houses with flood water damage were generally higher than outdoor bioaerosol concentrations regardless of the assessment method used. These results are the opposite of bioaerosol concentration trends typically observed in houses with no water damage. Total direct counts recovered more airborne bacteria, fungi and spores than did conventional plate counts. In this study, culturable methods significantly underestimated the quantity of airborne microorganisms both indoors and immediately outdoors of flood-damaged houses—at times this discrepancy was as large as 10^3 microorganisms/m^3.

Commercial air samplers have different collection efficiencies. They can significantly induce sampling stress affecting microbial recovery. The BioSampler consistently recovered a higher fraction of culturable bacteria and fungi than did an N6 Andersen impactor. Given that high efficiency liquid-capture offers capabilities for microscopy concurrent with culturing, and that sampling stress from liquid capture in swirling impingers is significantly lower, BioSamplers offer economical alternatives to impactor-based bioaerosol field studies with added benefits of extended sampling time and control over dilution factors (i.e. no upper detection limit).

The MVOC levels observed in the flood-damaged houses did not correlate with the bacterial and fungal bioaerosol concentrations measured (i.e. the house with the highest bioaerosol concentrations did not have the highest MVOC concentrations). However, given the relatively high air-exchange rates in the residences observed, the presence of MVOC levels indicated an indoor enrichment of microorganisms. While some VOCs are good indicators of microorganism growth, they could not be linked to a specific source or used to quantify the microorganisms from which they originate. The usefulness of MVOC as an index of airborne/surface associated indoor biological contamination may emerge as more studies provide a large enough database to establish VOC correlations to bioaerosol loads observed in the field.

Regardless of source, water can provide significant enrichment potential for microorganism growth on building materials not designed for such exposure, and this enrichment has been implicated to increase indoor bioaerosol levels. There is a lack of studies on bioaerosol exposures following the reoccupation of flood-damaged buildings; previous bioaerosol investigations of these common indoor environments are limited by the conventional culturing techniques used. Drying water-damaged material thoroughly and fast enough to prevent mold or bacterial growth is very difficult, particularly after large-scale water excursions associated with river floods. As part of this demonstration study, all of the houses monitored here were thoroughly cleaned prior to their reoccupation. It is likely that flood-impacted building components, although refurbished, were responsible for the elevated indoor bioaerosol concentrations observed herein. To help evaluate the long-term effectiveness of modern remediation practices, larger, multi-season residential flood surveys of indoor bioaerosol levels should be executed with direct measurements (microscopy, particulate matter and VOC) that provide expanded assessment capabilities complimentary to conventional culturing assays.

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