

# Assessment of Airborne Particles in Indoor Environments: Applicability of Particle Counting for Prediction of Bioaerosol Concentrations

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# ABSTRACT

Indoor bioaerosols have recently received considerable interest because of their impact on health. In this study, concentrations of bioaerosols in relation to airborne particulate matter in various indoor environments were investigated. The comparative performance of two common biosamplers, including the single-stage Andersen impactor and the all-glass impinger (AGI) for bioaerosol sampling, was also evaluated. The average levels of airborne bacteria and fungi sampled by Andersen were 516 and 176 colony forming units (CFU) m<sup>-3</sup> and by AGI were 163 and 151 CFU m<sup>-3</sup>, respectively. The highest bacterial levels were measured in residence apartments. The most predominant bacteria were belonged to *Staphylococcus* sp. and *Arthrobacter* sp. The Andersen impactor appeared to yield fungal concentrations that were comparable to the results obtained using the AGI biosampler. Meanwhile, Andersen impactor counts for bacteria were significantly higher than those obtained by AGI. Particle count data generated by the optical particle counter indicated that 95% of airborne particles were < 1  $\mu$ m in diameter. Statistical analysis revealed a significant correlation between particle counts of PM<sub>1</sub> and concentrations of culturable airborne bacteria measured with the both bioaerosol samplers.

Based on these results, the Andersen impactor performed much better than the AGI for sampling airborne bioaerosols in low-contaminated indoor environments. Accurate measurement of microbial concentrations in indoor environments should be performed by bioaerosol monitoring; however, combining particle counting with bioaerosol sampling could provide prompt information about rapid variations of air quality.

Keywords: Bioaerosol; Indoor; Andersen impactor; Impinger; Particle counting.

# INTRODUCTION

Interest in indoor air quality is booming. We spend a large portion of our time (about 90%) in a variety of enclosed environments (indoor) and therefore indoor air quality could significantly influence our general quality of life (Hospodsky *et al.*, 2012). Indoor air contains a mixture of airborne particles, including biological and non-biological aerosols (Kalogerakis *et al.*, 2005). A large number of studies have linked exposure to airborne particles, especially biological aerosols (bioaerosols), with a variety of negative effects (Mandal and Brandl, 2011; Duquenne *et al.*, 2013). Bioaerosols include bacteria, viruses, fungi, or their metabolites such as endotoxin. Bioaerosols come in a wide variety of sizes, shapes, and compositions depending on the source, aerosolization mechanisms, and environmental

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Tel.: 98-313-792-3278; Fax: 98-313-668-2509 *E-mail address:* nikaeen@hlth.mui.ac.ir conditions prevailing at the site (Heo *et al.*, 2014). Bioaerosols contribute to about 5% to 34% of indoor air pollution (Mandal and Brandl, 2011) and could cause many types of health problems including decreased lung function, respiratory symptoms, allergic diseases, asthma and rhinitis, infections and sick-building syndrome (SBS) (Mandal and Brandl, 2011; Duquenne *et al.*, 2013).

In recent years, several studies investigated the concentration of bioaerosols in various indoor environments (Kalogerakis *et al.*, 2005; Aydogdu *et al.*, 2010; Pegas *et al.*, 2010; Armadans-Gil *et al.*, 2013). However, bioaerosol levels in indoor environments depend on numerous physical and biological factors. Indoor bioaerosol concentration could also be affected by the construction material, housing type, and the life style of occupants (Mandal and Brandl, 2011). Therefore, assessment of microbiological quality of indoor air in different areas across the globe is necessary from a public health point of view, especially for protection of vulnerable groups such as children.

Several biosampler techniques are available for airborne biological particles monitoring, the two most common ones being the Andersen impactor and all-glass impinger (AGI) (Duquenne et al., 2013). A comparison of multiple samplers by Jensen et al. (1992) showed that the AGI, along with the Andersen microbial sampler, were the best samplers for collecting airborne bacteria. However, the sampling technique used may influence the results obtained and therefore, the choice of biosampler is critical to generating reproducible and accurate results (Mandal and Brandl, 2011). Very few studies have been performed to assess the comparability of these two methods for bioaerosol monitoring across a variety of indoor environments (Thorne et al., 1992; Armadans-Gil et al., 2013). On the other hand, microbiological air quality monitoring by biosampler techniques is time consuming and labor intensive. Some studies investigated the association between airborne biological particles and particle counts. Simple counting of particles of a certain size class might enable an approximation of indoor air microbial levels. However, inconsistent results have been published, with significant correlation in some cases and no relationship in others (Hargreaves et al., 2003; Agranovski et al., 2004; Haas et al., 2007; Cristina et al., 2012).

In order to address these challenges, this study was performed to 1) evaluate the concentration of bioaersols (bacteria and fungi) in various semi-arid indoor environments with two biosamplers (Andersen impactor and AGI), and to assess the relationship with particle counts; 2) determine the existence of endotoxin in indoor environments, and 3) identify the predominant bacteria in the samples.

#### **METHODS**

#### **Study Sites**

This study was designed to evaluate bioaerosol concentrations (bacteria and fungi) and test for the presence of endotoxin in 60 different indoor environments, including offices, laboratories, residential apartments, classrooms of primary schools, and university classrooms and dormitories in Isfahan, Iran. Isfahan is located in the center of Iran and has a semi-arid climate. Characteristics of sampling locations are presented in Table 1.

#### Air Sampling and Culture Media

Indoor airborne bioaerosol samples were collected using two types of biosampler simultaneously, including an Andersen impactor (N6 single-stage viable cascade impactor) with a flow rate of 15 L min<sup>-1</sup> for 5 min and an all-glass impinger (AGI) operated at a flow rate of 12.5 L min<sup>-1</sup> for 60 min to yield a sample volume of 750 liters. The measurements were performed during 9 months from September 2013 until May 2014. Bioaerosols were collected at a height of 1.5 m above the ground level to simulate the breathing zone. Outdoor concentrations were also measured during the study period.

The impactor was loaded with Petri dishes containing Tryptic Soy Agar (TSA) and Malt Extract Agar (MEA) for bacteria and fungi, respectively, prior to sampling. Duplicate sets of plates with each type of medium were taken at every sampling point, and the sampler surface was disinfected each time with a 70% ethanol solution.

A volume of 10 ml endotoxin-free water was used in the collection vessel of the impinger as the collection medium. After finishing each collection, the final volume of the impinger was measured and corrected for evaporation. All samples were transferred to the laboratory in an insulated box with cooling packs and processed immediately upon arrival in the laboratory. Aliquots of each collection medium were plated onto duplicate TSA and MEA plates.

For total bacteria analysis, the TSA plates were incubated at 30°C for 2–3 days and the incubation temperature for MEA plates was 25°C for 3–5 days of incubation. Colonies growing on both media were enumerated and calculated as colony-forming units per cubic meter (CFU m<sup>-3</sup>). Bacterial colonies were Gram-stained and characterized based on colony and cell morphology, and the abundance percentage of different types of colonies was recorded. Fungal colonies were also identified on the basis of colony and spore-morphological characteristics.

## Molecular Identification of Predominant Indoor Bacteria

Predominant indoor bacteria were isolated and subcultured on TSA agar plates. The isolated colonies were suspended in 100  $\mu$ l of deionized water, and genomic DNA was extracted by boiling for 15 min and centrifugation at 13,000 rpm for 5 min. The supernatant was used for PCR amplification using the Eubac 27F and 1492R primers, which amplify a ~1,420 bp fragment of the 16s rRNA gene as described by Farhadkhani *et al.* (2014). DNA sequencing of the amplified gene was performed, and DNA sequence

Sampling No		lo. of	Mean area	Mean volume	Number of	Type of cooling, heating		
location	n sa	imples	$(m^2)$	$(m^{3})$	occupants	and ventilation system		
Offices	5	10	18	65	1–3	Central HVAC <sup>*</sup> and Natural ventilation		
Laborator	ries	10	80	320	20-40	Central HVAC and Natural ventilation		
Resident apartmer	ial nts	10	20	56	3–4	Evaporative coolers, Radiators and Natural ventilation		
Primary sc classrooi	hool ns	10	25	75	2–25	Evaporative coolers, Radiators and Natural ventilation		
Universi classrooi	ty ns	10	40	160	2–45	Central HVAC and Natural ventilation		
Dormitor	ies	10	25	75	2-8	Evaporative coolers, Radiators and Natural ventilation		

Table 1. Characteristics of sampling locations

\* Heating, ventilating, and air conditioning.

analysis was undertaken by BLAST algorithms and databases from the National Center for Biotechnology Information (http://blast.ncbi.nlm.nih.gov /Blast.cgi).

#### **Endotoxin Detection**

Five milliliters of each impinger solution was transformed into a sterile pyrogen-free tube for endotoxin analysis. Samples were then stored at -25°C. An endotoxin test was carried out using Limulus Amebocyte Lysate (LAL) by the gel–cell method (Sigma).

#### **Particle Counting**

In this study an optical particle counter (GRIMM 1.109 dust monitor, Germany) was used to measure the numbersize distribution of aerosols. The number of particles with diameters between 0.25 and 32  $\mu$ m were counted and then expressed in particles m<sup>-3</sup> in the range of PM<sub>0.5</sub>, PM<sub>1</sub>, PM<sub>2.5</sub> and PM<sub>10</sub>.

## Meteorological Conditions

During the bioaerosol sampling, environmental parameters including temperature (°C) and relative humidity (RH, %) were also monitored and recorded using a portable weather station (Kimo) at each sampling location.

#### Statistical Analysis

Statistical analyses were performed with SPSS 20.0. Kolmogorov-Smirnov's normality tests were performed for identifying the use of parametric vs. non-parametric tests. For comparison of groups the Mann–Whitney test was applied. Spearman's rank correlation coefficient was used to determine correlation coefficients between the analyzed parameters. All probability (P) values smaller than 0.05 were considered statistically significant for all analyses.

# RESULTS

# Indoor and Outdoor Bioaerosol Concentration

The mean concentration of airborne bacteria and fungi in different indoor environments as measured by the AGI sampler and the Andersen impactor are shown in Table 2.

In both sampling methods, the highest bacterial level was measured in residence apartments, while the lowest level was seen in offices. In apartments, bacterial counts ranged from 132 to 2678 CFU m<sup>-3</sup> (mean, 944 CFU m<sup>-3</sup>) and 50 to 1060 CFU m<sup>-3</sup> (mean, 214 CFU m<sup>-3</sup>) with the Andersen

impactor and the AGI sampler, respectively. As can be seen in Table 2, the highest level of fungi was seen in offices with an average 216 CFU m<sup>-3</sup> and 203 CFU m<sup>-3</sup> for the Andersen impactor and the AGI sampler, respectively. The lowest fungi level was observed in university classrooms by both sampling methods.

For outdoor samples, mean bacterial and fungal concentrations were measured as 342 CFU  $m^{-3}$  and 107 CFU  $m^{-3}$  with the Andersen impactor, respectively.

Statistical analysis showed that the fungal concentrations detected by the two sampling methods were not significantly different, but for bacteria, the concentrations measured by the AGI were significantly lower than that detected by the Andersen impactor.

# Characteristics of Bioaerosols and Predominant Indoor Bacteria

Gram-positive bacteria were observed to be the predominant bacteria in all samples and were present in 80% (43% bacilli, 37% cocci) and 63% (36% bacilli, 27% cocci) of the samples collected by the Andersen impactor and the AGI sampler, respectively. In both sampling methods, the most common fungi included dematiaceous fungi (mostly *Alternaria* sp. and *Cladosporium* sp.), *Penicillium* sp., yeasts, and *Aspergillus* sp., which were isolated from 52%, 47%, 45%, and 30% of the Andersen impactor samples, respectively. With regard to the AGI samples, this order was as follows: dematiaceous fungi (43%), *Penicillium* sp. (24%), yeasts (20%), and *Aspergillus* sp. (17%). Based on 16s rRNA gene sequence analysis of predominant bacteria in indoor air, fourteen bacterial species from six genera were identified (Table 3).

#### **Endotoxin Detection**

Endotoxin analysis showed that about 68.3% (41/60) samples were positive. The proportions of endotoxin-positive samples in different indoor environments are shown in Fig. 1. The highest proportion of endotoxin-positive samples was observed in university classrooms (90.9%). Statistical analysis showed that the bacterial levels in samples collected by the AGI sampler differed significantly between endotoxin-negative and endotoxin-positive samples.

#### **Particle Counting**

The highest number of counted particles was in the size range of  $PM_{0.5}$ . Table 4 shows the distribution of particle numbers in the four particle size channels.

**Table 2.** Mean (maximum) microbial concentrations (CFU  $m^{-3}$ ) in bioaerosol samples collected using the AGI and Andersen samplers in indoor environments.

Sampling logation	Bac	teria	Fungi		
Sampling location	AGI	Andersen	AGI	Andersen	
Offices	64 (288)	201 (622)	203 (361)	216 (489)	
Laboratories	126 (1120)	309 (667)	125 (298)	154 (420)	
Residential apartments	214 (1060)	944 (2678)	182 (1060)	190 (356)	
School classrooms	183 (324)	430 (900)	145 (325)	187 (367)	
University classrooms	178 (480)	395 (889)	99 (467)	126 (388)	
Dormitories	211 (720)	816 (2444)	152 (183)	186 (300)	
Total	163 (1120)	516 (2678)	151 (1060)	176 (489)	

Genus	Bacterial species	Accession number in GenBank		
Rothia	Rothia sp.	KU184510		
Vegeuria	Kocuria rosea	KU184511		
косина	Kocuria carniphila	KU184512		
	Arthrobacter globiformis	KU184509		
Arthrobacter	Arthrobacter oxydans	KU184513		
	Arthrobacter citreus	KU184514		
Mieneeseens	Micrococcus luteus	KU184516		
Micrococcus	Micrococcus lylae	KU184518		
Bacillus	Bacillus cereus	KU184521		
	Staphylococcus warneri	KU184515		
	Staphylococcus hominis	KU184517		
Staphylococcus	Staphylococcus epidermidis	KU184520		
	Staphylococcus arlettae	KU184522		
	Staphylococcus equorum	KU184523		





Fig. 1. The proportion of endotoxin-positive samples in different indoor environments.

# **Environmental Parameters**

The indoor ambient temperature ranged from 16°C to 28°C, with a mean value of 21.9°C, and the relative humidity ranged from 14% to 56%, with an average of 26.9%. Spearman correlation analysis showed no significant correlation between environmental parameters and bacterial concentrations in samples collected by AGI sampler. Meanwhile, there was a weak correlation between temperature and bacterial levels in samples collected by the impaction method. Similarly, a weak correlation was seen between RH and bacterial levels. A positive correlation was also observed between RH and concentration of fungi by both sampling methods (Table 5).

# Association between Bioaerosol Concentrations, Particle Counts, and Environmental Parameters

In order to identify the potential association between the parameters analyzed, a correlation analysis was performed, and the results are presented in Table 5. A positive correlation was only found between indoor bacterial levels and particle counts of  $PM_{0.5}$  and  $PM_1$  by both methods of sampling. However, there was no correlation between indoor fungal concentrations and particle counts for each range.

The scatter plots in Fig. 2 show the relationship between  $PM_1$  particle counts and the concentration of bacteria by the AGI sampler and the Andersen impactor.

# DISCUSSION

Exposure to bioaerosols has become a significant public health concern. However, no international standard is available with regards to acceptable maximum bioaerosol levels in indoor environments. In this study, the average bacterial level detected by the Andersen impactor in residence apartments and dormitories was nearly twice that stipulated by the WHO guidelines (500 CFU  $m^{-3}$ ), varying in the range

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Sampling Location	$PM_{10}$	PM <sub>2.5</sub>	$PM_1$	PM <sub>0.5</sub>
Offices	338564	337253	316479	303460
Offices	(152890-605100)	(152694–604764)	(151243-602564)	(150077-600693)
Laboratorias	175631	174110	171092	165224
Laboratories	(61947–635543)	(61906–635486)	(61305–634513)	(60570-632298)
Desidential enertments	166201	166074	158933	152371
Residential apartments	(76543-233285)	(76452-233281)	(75445-232982)	(73763-232175)
Drimory school classrooms	389340	389107	373964	362114
Primary school classioollis	(80260-1267841)	(80255-1267812)	(80119-1267084)	(79765-1265048)
I Iniconsity alongue and	307057	307014	295419	282165
University classioonis	(197387-777675)	(197373-777608)	(197122-776678)	(196362-774575)
Dormitorios	290815	290106	275093	262181
Dormitories	(94083-1361012)	(94068-1360995)	(93357-1360440)	(91387–1353899)
Total	277935	277278	265163	254586

**Table 4.** Mean (range) of particle numbers ( $\times 10^3$  particles m<sup>-3</sup>) in the four particle size ranges.

Table 5. Correlation matrix of the analyzed parameters in indoor environments.

	Bacteria (Andersen)	Fungi (Andersen)	Bacteria (AGI)	Fungi (AGI)	PM <sub>0.5</sub>	$PM_1$	T <sup>a</sup>	$\mathrm{H}^{\mathfrak{b}}$
Bacteria (Andersen)	1							
Fungi (Andersen)	-0.077	1						
Bacteria (AGI)	$0.601^{**}$	-0.099	1					
Fungi (AGI)	-0.009	$0.52^{**}$	0.082	1				
PM <sub>0.5</sub>	$0.705^{**}$	-0.149	$0.52^{**}$	-0.116	1			
$PM_1$	$0.825^{**}$	-0.152	$0.65^{**}$	-0.124	$0.98^{**}$	1		
Т	$0.32^{*}$	-0.17	0.165	-0.01	0.096	0.11	1	
RH	$0.30^{*}$	0.43*	0.08	$0.28^{*}$	0.008	0.02	0.12	1

<sup>a</sup> Temperature; b: Relative humidity.

\* Correlation significant at the 0.05 level (2-tailed).

\*\* Correlation significant at the 0.01 level (2-tailed).



Fig. 2. Scatter plots of relationship between particle counts of  $PM_1$  and bacterial concentrations in samples collected by the Andersen impactor and AGI.

of 33–2678 CFU m<sup>-3</sup> (Table 2) (WHO, 2002). Similarly, Lee *et al.* (2006) reported on geometric mean bacterial values in homes ranging between 10 and  $10^3$  CFU m<sup>-3</sup>. Our results showed that in school classrooms, bacterial counts ranged from 68 to 900 CFU m<sup>-3</sup>. In a study by Pegas *et al.* (2010), bacterial counts exceeded 500 CFU m<sup>-3</sup> in all studied schools (934–1634 CFU m<sup>-3</sup>). A study by Mentes *et al.* 

(2009) using a single-stage Andersen sampler showed that the bacterial levels of kindergartens (mean, 1251 CFU m<sup>-3</sup>) and primary schools (mean, 1131 CFU m<sup>-3</sup>) were higher than in other environments, highlighting the need for remedial action favoring the children's health.

Principal factors affecting the level of indoor airborne microorganisms may include the extent of human activity,

population density, and ventilation efficiency. Although classrooms being generally more crowded, higher concentrations of airborne bacteria in residential homes and dormitories detected in our study may be due to house characteristics, including increased insulation of buildings, hence deficient in fresh air, and/or weakly maintained or operated ventilation systems (Srikanth *et al.*, 2008). Comparison of indoor to outdoor bacterial concentrations showed higher levels in indoor samples than outdoor. This result indicated that indoor bioaerosol sources can be a cause of higher indoor concentrations (Nasir *et al.*, 2012).

The total airborne fungal concentration ranged from 50 to 1060 CFU m<sup>-3</sup> in various indoor environments. Higher fungal concentrations were observed in offices and residence apartments (Table 2), but the concentrations were generally lower than those reported in other studies. Lee et al. (2006) reported that the concentration of airborne fungi in six Cincinnati homes was typically between 0 and 1362 CFU  $m^{-3}$ . Other studies have shown fungal concentrations ranging between 103 and 1116 CFU m<sup>-3</sup> in offices (Chao et al., 2002; Mentese et al., 2009; Bonetta et al., 2010). Higher fungal levels (463-3125 CFU m<sup>-3</sup>) have been observed in residence places, which may promote fungal growth due to high relative humidity (Hargreaves et al., 2003; Haas et al., 2007). However, our research area as a semi-arid area has a low relative humidity and low numbers of fungi could be related to this factor. For outdoor experiments, lower concentration of fungi was observed than indoor. In the study of Nasir et al. (2012), the concentrations of both bacterial and fungal aerosols were higher outdoors than indoors at both rural and urban sites.

In the current study, gram-positive bacteria dominated in all samples. Previous studies have also demonstrated that gram-positive bacteria are the most commonly found airborne bacteria in indoor environments (Zhu et al., 2003; Aydogdu et al., 2010). The relatively low presence of airborne gramnegative bacteria may primarily reflect the short survival periods of such bacteria in the airborne state; meanwhile, gram-positive cells have a fairly hard and protective cell envelope. Based on 16S rRNA gene sequence analysis of predominant bacteria in indoor air, fourteen bacterial species from six genera were identified (Table 3). Staphylococcus, Micrococcus, Bacillus, and Kocuria are the most common bacteria found in indoor environments (Gorny and Dutkiewicz, 2002; Mentese et al., 2009), and Arthrobacter has also been seen in some indoor environments. Some of these bacteria such as Staphylococcus and Micrococcus have human origin (Mandal and Brandl, 2011). Bacillus sp., Arthrobacter globiformis and Staphylococcus warneri are known to have allergenic or immune toxic effects on human health (Mandal and Brandl, 2011).

Predominant fungal species included *Alternaria*, *Cladosporium*, *Penicillium*, and *Aspergillus*, respectively. Similarly, the most commonly reported fungal species in indoor environments are *Alternaria*, *Aspergillus*, *Cladosporium*, and *Penicillium* (Zorman and Jeršek 2008; Bernasconi *et al.*, 2010). Exposure to indoor fungi such as *Alternaria*, *Aspergillus*, and *Penicillium* can exacerbate symptoms of asthma and allergic rhinitis in susceptible individuals (Portnoy *et al.*, 2005). For individual fungi, the threshold concentration for evoking allergic symptoms has been estimated to 100 *Alternaria* spores per cubic meter. A concentration of *Aspergillus* spores above 50 CFU m<sup>-3</sup> has also been associated with a higher prevalence of sick building syndrome (Chen *et al.*, 2010). In our study, the maximum concentration of *Aspergillus* was 160 CFU m<sup>-3</sup> and seen in offices, representing a potential health risk for exposed individuals.

A comparison of the performance of the Andersen and the AGI bioaerosol sampler showed that the Andersen sampler yielded higher bioaerosol counts in all indoor environments tested. However, the Andersen impactor appeared to reveal fungal concentrations comparable to the results achieved using the AGI biosampler. Meanwhile, the Andersen counts for bacteria were significantly higher than the counts obtained by the impinger. At present, no standardized method presented for collection of bacterial and fungal bioaerosols exists. Several factors affect microbe collection and survival in bioaerosol samplers and hence the accuracy of enumeration. In comparison, the impingement method may not be as suitable for fungal bioaerosols as the impaction method due to the hydrophobic nature of many fungal spores (Cage et al., 1996). Our results showed that the Andersen impactor was also more efficient in terms of capturing bacterial aerosols. Re-aerosolization of bacteria during sampling by an impinger may result in an under-estimation of bacterial concentration (Jensen and Schafer, 1998) and a decrease in precision. In contrast, Thorne et al. (1992) founded that microbe sampling by the Andersen method had a poor data yield for bacteria in an environment with high bioaerosol concentrations because of plate overloading. Therefore, the selection of an appropriate method should depend on the expected bioaerosol concentrations and environmental conditions.

In an exposure assessment, the use of concentration of culturable bacteria and fungi may not reflect the health risks sufficiently well, since microbial fragments such as endotoxin can also cause adverse health effects (Loftness *et al.*, 2007). Endotoxin is therefore used as an indicator for assessment of indoor air quality. The present study revealed the presence of endotoxin in 68% of the indoor environment samples (Fig. 1). According to the detection limit of endotoxin analysis (0.5 EU ml<sup>-1</sup>) and collected volume of air samples, the airborne endotoxin levels were higher than 4-5 EU m<sup>-3</sup> in positive samples. Douwes *et al.* (2003) have proposed a no observed effect level (NOEL) as 20 ng m<sup>-3</sup> for endotoxin. However, dose-response relationship for endotoxin exposure has not been established yet.

Although, endotoxin is associated only with presence of gram-negative bacteria but, our statistical analysis showed a significant difference between bacterial levels detected by the impingement method in endotoxin-positive and negative samples.

The correlation analysis between bioaerosol concentrations and particle counts revealed a significant correlation between particle counts of  $PM_{0.5}$  and  $PM_1$  and the concentration of culturable airborne bacteria as measured with both bioaerosol samplers (Table 5). Parat *et al.* (1999) also found a correlation between concentrations of culturable bacteria and particle counts. Particle count data indicated that 95% of the aerosols were  $< 1 \mu m$  in diameter (Table 4). Although, many bacteria have sizes  $< 1 \mu m$ , the percentage of airborne bacteria detected by the Andersen method in relation to the total number of airborne particles (< 1  $\mu$ m) detected by the particle counter was 0.2%. Thus, particle counts could not be considered as a direct measure method for evaluation of airborne bacteria. However, integrating particle counting with bioaerosol monitoring may help to evaluate individual exposure to airborne bacteria, by instantaneous detection of rapid variations that could go undetected by periodic microbial measurements (Parat et al., 1999). On the other hand, correlation coefficients were found to be higher with the Andersen biosampler when compared with the AGI biosampler (Table 5). The higher correlation between particle counts and bacterial concentrations detected by the Andersen method could reflect a better evaluation of airborne bacteria by this biosampler.

This study showed no statistically significant association between fungal levels and particle counts (Table 5). Similarly, a study by Hargreaves *et al.* (2003) showed no statistically significant association between concentrations of fungal spores and particle concentrations in indoor environments of 14 residential suburban houses in Brisbane, Australia, but fungal colony counts correlated well with total number of particles  $< 2.5 \ \mu m$ . Armadans-Gil *et al.* (2013) showed a relationship between the concentrations of particles  $\ge 0.5 \ \mu m$ and particles  $\ge 1 \ \mu m$  and airborne fungi in hospital rooms.

Environmental factors such as temperature and relative humidity could influence bioaerosol concentrations. In the present study, relative humidity has a significant effect on fungal concentrations detected by the two methods. However, there was a weak but significant positive correlation between temperature and bacterial counts by impaction sampling. Similar results were shown in studies finding a significant association between bioaerosol concentrations and environmental factors, such as temperature and relative humidity (Gorny and Dutkiewicz, 2002; Zhu et al., 2003; Nikaeen et al., 2009). Frankel et al. (2012) found that indoor temperature and RH were positively correlated with levels of airborne fungi in homes in the northeast United States, and indoor temperature was negatively correlated with levels of bacteria in Cincinnati residences.

### CONCLUSIONS

Bioaerosol concentrations varied with sampling environment, and the highest bacterial levels were observed in residence apartments. The Andersen sampling method yielded higher bioaerosol concentrations than the AGI sampler in all environments tested. It appears that the Andersen biosampler yields more representative results of bioaerosol concentrations and being a simple and easy method, is more suitable for indoor areas where bioaerosol loads are fairly low. However, in order to more accurately evaluate the effectiveness of these collection methods, bioaerosol samples would need to be taken in an experimental environment with known concentration of airborne bacteria and fungi. Our results also showed that particle counting could not substitute bioaerosol measurements, but combining it with bioaerosol monitoring may enable instantaneous detection of rapid variations, which are not measurable by periodic bioaerosol monitoring.

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