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Assessment of microbiological indoor air quality in public buildings: A case study (Timisoara, Romania)

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The indoor air quality in public buildings is essential for the health of employees and visitors. To investigate the potential influence of airborne germ loads on human health, two sampling campaigns were conducted during 2009 in several public buildings in Timisoara (Romania). The quality of air revealed highly significant differences among different sites. Cluster analysis accurately classified the investigated buildings into three main groups and for most groups of aerial microorganisms, the measured values rarely fell below the normal concentrations in indoor environments. Although the structure of airborne flora varied widely among different locations, mesophilic bacteria and molds were the main determinants of indoor air quality in investigated buildings. It was found that these two factors explained over 90% of the overall average dissimilarity in the structure of indoor airborne microbiota. Moreover, multivariate analysis showed a strong positive relationship between these two factors, which is probably related to the number of daily occupants and visitors as well as to the building age. Further research is required to determine more accurately the relationship between ventilation performance and air quality.

Key words: Indoor air quality, airborne germ load, public buildings, mesophilic bacteria, airborne molds.

INTRODUCTION

The air quality in indoor environments has attracted research interest during the past decades or so (Jones, 1999). Most problems related to indoor air quality result from complex interactions among building occupants, indoor environment (inadequate temperature, excessive humidity), insufficient outdoor air intake, building materials and furnishing, and air contaminants (chemicals, bacteria, molds, vapors) (Yu et al., 2009). Airborne bacteria are ubiquitous in the earth's atmosphere, and originate from numerous sources, such as lakes, oceans, soils, humans, and animals (Bowers et al., 2012). A recent study revealed more than 1,800 types

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of bacteria in air samples taken from the Texas cities of San Antonio and Austin, with some of them posing a serious public health hazard (Brodie et al., 2007). Airborne fungal contaminants (molds, yeasts, mushrooms) present a similar threat to humans, being occasionally associated with dangerous infections and toxicity (Łukaszuk et al., 2011). Therefore, the airborne germ load in indoor environments is essential for the health of employees and visitors (Yu et al., 2009).

Indoor air quality (IAQ), as the name implies, is a term used to assess the quality of the air in offices and other building environments (Fanger, 2000). Determination of IAQ relies on collection of air samples, monitoring human exposure to pollutants, collection of samples on building surfaces, and computer modeling of air flow inside buildings (Klepeis, 2006). There is strong evidence suggesting that airborne microbiota greatly affect IAQ. For example, the sick building syndrome (SBS), which includes a large variety of nonspecific symptoms that occurs in the residents of a building (Joshi, 2008), is frequently related to elevated levels to which airborne microorganisms occur in indoor air in typical enclosed spaces (Teeuw et al., 1994; Fischer and Dott, 2003). In this context, this study aimed at assessing IAQ in several important public buildings from Timisoara (Timis county, Romania). To our knowledge, the present work is the largest survey from the Western Romania which investigates the occurrence of airborne bacterial and fungal species in indoor environments. The results provide industrial hygienists, allergists, and other public health practitioners with comparative information on the most common airborne germs in public buildings in the investigated areas, thus allowing a reliable assessment of microbiological risks that IAQ may pose on visitors' and occupants' health.

MATERIALS AND METHODS

Air sampling

The different types of airborne germs selected for inclusion in this study were chosen on the basis of being frequently related to various airborne diseases. Air samples were collected by using a microbiological air sampler MAS-100 Eco. This device can aspire 100 L of air per minute and collect for each air sample about 1,000 L of air. The air aspiration speed, which is the speed at which the airborne microorganisms hit the agar surface, is about 11 m per second; this speed enables all particles > 1 µm to be collected inside the air sampler. This device functions properly from 0 to 40°C, and for a relative humidity ranging from 0 to 80%, respectively. The air flux carrying the airborne particles is directed toward the Petri dishes located inside the air sampler, which contain elective culture media for each group of investigated microorganisms.

Two air sampling campaigns were conducted throughout the experimental period; the first campaign lasted from September 2009 to October 2009 and the second campaign from November 2009 to December 2009. For each sampling campaign, the samples were collected in triplicate in five different public locations from the city of Timisoara: Timisoara City Hall (abbr. PMT), Timis County House of Pensions (abbr. CJT), The Direction for People Evidence from Timisoara (abbr. DEVT), The Direction for Work and Social Protection from Timisoara (abbr. DMPST), and The Direction for Community and Social Assistance from Timisoara (abbr. DASCT). Whenever it was possible, for each investigated building, we collected data on daily building occupants, visitor traffic, building age, total number of rooms and offices, total covered area, ventilation and air conditioning systems.

Culture media

The microorganims were grown on elective culture media: (1) Plate-Count Agar P (CA/Agar) for mesophilic bacteria; (2) blood agar (Gelose + 5% defibrinated ram blood) for alfa-hemolytic streptococcci and beta-hemolytic streptococci; (3) BEA/Agar (Bile-Esculin-Azide) for enterococci; (4) agar blood agar (Gelose + 5% defibrinated ram blood) for *Bacillus cereus*; (5) Brilliance TM ESBL Agar for *Escherichia coli* and coliform bacteria; (6) MacConkey Agar for other gram-negative bacili; (7) PYO (Selected *Pseudomonas aeruginosa* agar) for *Pseudomonas aeruginosa*; (7) Sabouraud agar with chloramphenicol and gentamicin (SCG) for molds; (8) CandiSelect^{TM} 4 for *Candida sp*. yeasts. Most culture media were purchased from BioRad (Berkeley, USA) that is PCA, BEA, BP, Blood Agar, Mac Conkey Agar, Sabouraud Agar, CandiSelect^{TM} 4, except Brilliance medium which was purchased from Oxoid (Basingstoke, UK),that is Brilliance medium. In addition, specific biochemical analyses were conducted to confirm the presence of *E. coli*, *Escherichia vulneris*, *Enterobacter cloacae*, *Citrobacter freundii*, *Pantoea* ssp., *P. aeruginosa*, *Stenotrophomonas maltophilia* and *Sfingobacterium multivorum*.

Statistical analysis

All the data were analyzed using Statistica 10 and Past statistical softwares (Statistica 10; Hammer et al., 2001). The principal component analysis (PCA) was used to find an appropriate approach for combining variables into a small number of subsets. A Similarity Percentage Analysis (SIMPER) using the Bray-Curtis similarity measure and 9,999 permutations was performed to determine which group of airborne microorganisms accounted for most dissimilarities observed among investigated microbiotas. This statistical method is routinely used in environmental risk assessment for determining which taxa are primarily responsible for the observed differences among different groups of samples (Clarke, 1993).

The next step tested the overall significance of these differences by using a one-way analysis of similarity (ANOSIM). This approach generates a global value of R that ranges between +1 and -1; a value of 0 shows no differences among the samples. Post hoc comparison of pair-wise R values defines on a scale of 0 (indistinguishable) to 1 (all similarities within groups are less than any similarity between groups) differences existing between groups: $R > 0.75$ as well separated groups; $R > 0.5$ as overlapping groups; $R < 0.25$ as barely separable groups (Clarke and Gorley, 2001). Finally, a cluster analysis using the Bray-Curtis similarity measure was implemented to classify the investigated sites depending on airborne germ load. A $p < 0.05$ was considered as significant.

RESULTS

PMT building, which was built in 1929, has 107 rooms and covers a total area of 2,817.23 m^2. The heating during winter and air-conditioning during summer are

Table 1. Average values of airborne microorganisms depending on location[1].

Airborne microorganism	Site				
	PMT	CJT	DEVT	DMPST	DASCT
Mesophilic bacteria	378.00 ± 250.05	490.00 ± 70.71	835.00 ± 205.06	270.00 ± 98.99	130.00 ± 14.14
Staphylococcus aureus	1.00 ± 3.16	20.00 ± 28.28	-	10.00 ± 14.14	-
Alfa-hemolytic streptococci	1.00 ± 3.16	-	5.00 ± 7.07	5.00 ± 7.07	-
Beta-hemolytic streptococci	-	-	-	-	-
Enterococci	-	15.00 ± 21.21	-	-	-
Bacillus cereus	5.00 ± 8.49	15.00 ± 7.07	5.00 ± 7.07	-	5.00 ± 7.07
Escherichia coli	-	5.00 ± 7.07	-	-	-
Coliform bacteria	-	5.00 ± 7.07	5.00 ± 7.07	-	-
Other gram-negative bacteria	1.00 ± 3.16	35.00 ± 49.49	-	5.00 ± 7.07	-
Psudomonas aeruginosa	-	-	-	5.00 ± 7.07	-
Yeasts	-	-	-	-	-
Molds	227.00 ±197.99	680.00 ± 28.80	680.00 ± 28.80	200.00 ± 56.56	120.00 ± 56.56

[1]All the data are expressed as CFU/m^3; values of mean ± standard deviation.

Table 2. Selected data on the admitted levels of airborne microorganisms in indoor environments.

Airborne microorganism	Normal level (CFU/m^3)	Contamination level (CFU/m^3)
Mesophilic bacteria	Clean air: < 4500 (winter)	Impure air > 7000 (winter)
	Clean air: < 1500 (summer)	Impure air > 2500 (summer)
Alfa-hemolytic streptococci	Clean air: < 36 (winter)	Impure air > 124 (winter)
Beta-hemolytic streptococci	Clean air: < 16 (summer)	Impure air > 36 (summer)
Yeasts	Clean air: < 550	Medium infestation: 550-770
Molds		Maximal infestation > 770

performed by using a convective ventilation system mounted in the walls of offices and halls. CJT building was built in 2002 and covers a total area of 2,388 m^2. The average number of daily visitors was 75, whereas the air ventilation was achieved by air conditioning. DASCT building has 11 rooms and covers a total area of 289.33 m^2; the average number of visitors was 250-300 per week, whereas the ventilation inside the building was performed by using air-conditioning devices. However, no similar information was available for DEVT and DMPST buildings.

Table 1 gives the mean values (with standard deviations) for parameters of air quality, whereas Table 2 shows the levels of airborne microorganisms over which IAQ can be considered potentially dangerous for human health. The highest levels of mesophilic bacteria in indoor air were shown to occur at the site DEVT. The most diversified airborne microbiota was reported for the site CJT, and included various species, such as *S. aureus*, *Stenotrophomonas maltophilia*, *Sphigobacterium multivorum*, *B. cereus,* or *E. coli*. However, as shown in the Tables 1 and 2, the measured levels did not exceed the normal concentrations for mesophilic, alfa- and beta-hemolytic bacteria in indoor air, irrespective of location. Airborne mold load generally varied within the normal range for this parameter of air quality, except for the site CJT, wherein the measured values showed a medium level of air contamination (Tables 1 and 2). In addition, the latter location revealed the highest concentration for *S*. *aureus*, enterococci, *B. cereus*, and other gram-negative bacilli (Tables 1 and 2).

Principal component analysis (PCA) showed that the PC1 and PC2 accounted for almost 100% of the total variance for the 12 predetermined variables. PC1 explained 77.11% of the total variance, and showed high positive loadings for airborne mesophilic bacteria and mold levels (Table 3). PC 2 accounted for 22.55% of the total variance, and revealed a negative relationship between the mesophilic bacteria and mold levels in indoor air (Table 3).

The results of SIMPER analysis showed that the structure of indoor airborne flora varied widely, depending on location (overall average dissimilarity: 41.74%). Mesophilic bacteria and molds were the best discrimating

Table 3. Canonical loadings for the first three principal components.

Air microorganism	Principal component		
	PC 1	PC 2	PC3
Mesophilic bacteria	**0.82***	**-0.57***	0.01
Staphylococcus aureus	0.01	0.03	-0.36
Alfa-hemolytic streptococci	0.00	-0.01	0.01
Beta-hemolytic streptococci	0.00	0.00	0.00
Enterococci	0.01	0.02	0.37
Bacillus cereus	0.01	0.01	0.11
Escherichia coli	0.00	0.01	0.12
Coliform bacteria	0.00	0.00	-0.08
Other gram-negative bacteria	0.02	0.05	0.84
Psudomonas aeruginosa	0.00	0.00	0.00
Yeasts	0.00	0.00	0.00
Molds	0.57*	0.82*	-0.04

*Bold values express significant canonical loadings

Table 4. Mean abundance for each investigated taxon depending on location.

Taxon	Contribution	Cumulative %	Mean abundance				
			PMT	CJT	DEVT	DMPST	DASCT
Mesophilic bacteria	23.13	55.41	378	490	835	270	130
Molds	15.52	92.59	227	680	205	200	120
Other gram-negative bacteria	0.88	94.71	1	35	0	5	0
Staphylococcus aureus	0.73	96.47	1	20	0	10	0
Bacillus cereus	0.51	97.71	5	15	5	0	5
Enterococci	0.31	98.46	0	15	0	0	0
Coliform bacteria	0.20	98.95	0	5	5	0	0
Psudomonas aeruginosa	0.19	99.42	0	0	0	5	0
Alfa-hemolytic streptococci	0.13	99.75	1	0	5	0	0
Escherichia coli	0.10	100.00	0	5	0	0	0
Yeasts	0	100.00	0	0	0	0	0
Beta-hemolytic streptococci	0	100.00	0	0	0	0	0

groups of airborne microorganisms, accounting for most of the overall average dissimilarity (Table 4). The other taxons, by contrast, contributed to less than 10% of the overall average dissimilarity (Table 4). In addition, it wasfound that the main discriminant of air quality pattern in investigated buildings is the ratio between mesophilic bacteria and mold loads. This parameter was shown to be supraunitary for all locations, except for the CJT site. Moreover, the indoor air quality in investigated buildings from Timisoara showed highly significant differences concerning total airborne germ loads among sites (Global $R = 0.821$, $p = 0.001$). The quantitative population dynamics in indoor air were different in most locations ($R = 1$, $p > 0.333$), in contrast with those found between the site DMPST and either the site PMT or DASCT, which tended to overlap in taxon frequencies ($R \leq 0.5$, $p > 0.333$).

Cluster analysis using constrained Ward's method (Figure 1) showed that, based on the indoor air quality, the investigated locations can be classified into three main groups. The first group corresponded to the locations with the highest concentrations of aerial microorganisms (CJT and DEVT). The second group contained the sites wherein intermediate levels of airborne germs were reported (PMT, DMPST), whereas the last group included the cleanest area in terms of airborne germs loads (DASCT).

DISCUSSION

Microorganisms are well adapted to aerial transmission through nasopharyngeal secretions and saliva drops, and can easily survive dehydration; therefore, they can be

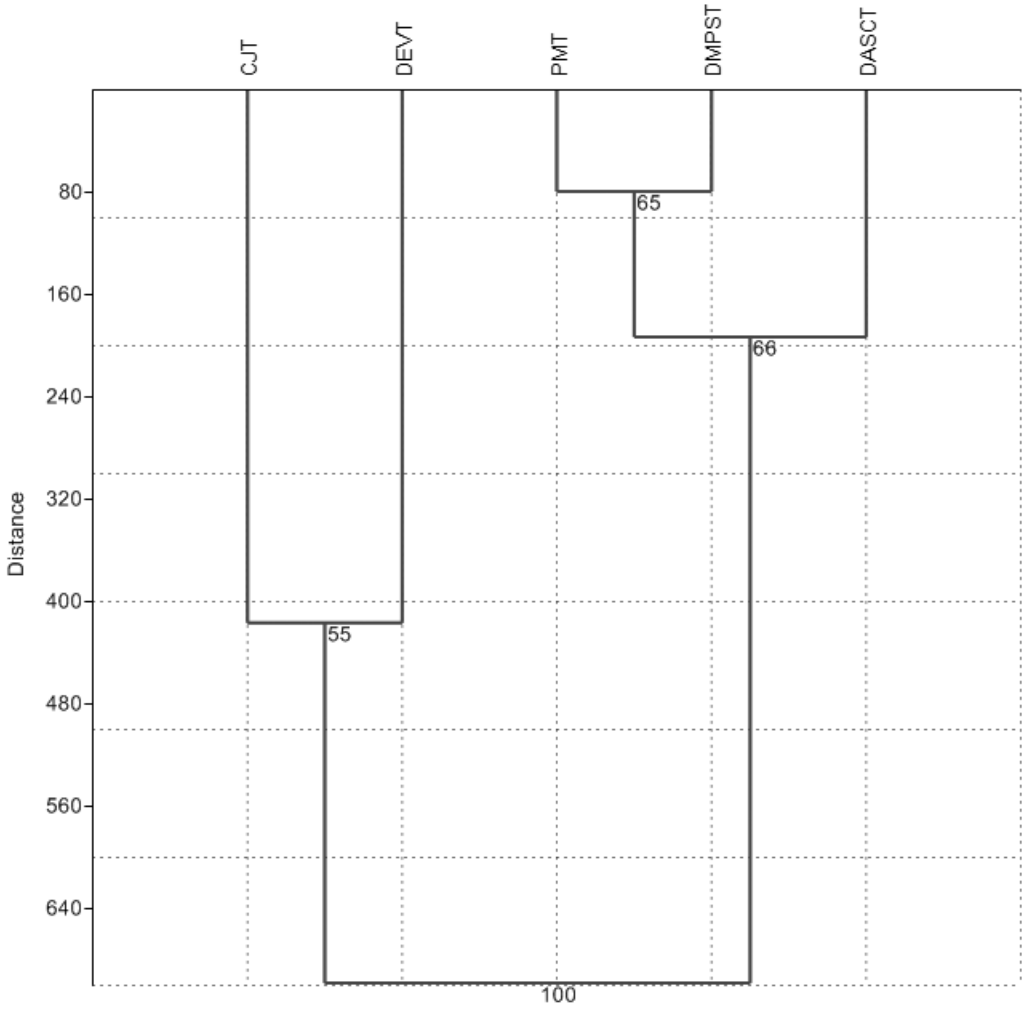

Figure 1. Cluster analysis of investigated locations.

easily transmitted from one host to another (Brooks et al., 1998). Although *S. aureus* is a part of normal skin and nasal passages flora, it can cause a large range of illnesses (Kluytmans et al., 1997; Cole et al., 2001), from minor skin infections (furuncles, pimples, impedigo, abscesses) to life-threatening diseases (pneumonia, meningitis, sepsis) (Mandell et al., 2000; Murray et al., 2007; Biuc et al., 2008). Airborne microorganisms affect human health, especially generating respiratory allergies, and infectious lung diseases (Fracchia et al., 2006). *B. cereus* is generally associated with food borne illnesses, causing severe nausea, vomiting, and diarrhea (Kotiranta et al., 2000). Bacteria belonging to *Enterococcus* genus are potentially related to various types of infections such as urinary tract infections, bacteremia, or meningitis (Fisher and Phillips, 2009). Among alfa-hemolytic streptococci, *S. pneumoniae* is the main cause of bacterial pneumonia. Beta-hemolytic streptococci are the causative agents in a wide range of streptococcal infections (streptococcal amigdalytis and pharyngitis, toxic shock syndrome, meningitis in neonates) with the most common representatives being *S. pyogenes* and *S. agalactiae* (Mandell et al., 2000; Murray et al., 2007; Biuc et al., 2008). Fungal exposure can result in skin and breathing irritations, and even cause dangerous infection and toxicity (Fung and Hughson, 2003). In a recent study, Lou and collaborators isolated *Penicillium*, *Cladosporium*, *Alternaria*, and *Aspergillus* in air samples from university campuses, and concluded that airborne fungi may cause a number of allergic, inflammatory, and toxic reactions in humans (Lou et al., 2012). These information clearly show the serious hazards that airborne microbiota may pose to human health.

A recent study revealed log-linear relationships between the amount of dust and either Gram-negative or mesophilic bacteria load in air (Schlosser et al., 2009). The concentrations of airborne mold spores were shown to be associated to damp buildings (Husman, 1996). There-

fore, one may expect that air quality at the site CJT was lower than in the other locations due to the higher air humidity and dust load. A possible explanation for the elevated levels of mesophilic bacteria that are found at the site DEVT is related to the fact that this site is located near the Timisoara Municipal Hospital.

In this study, we found a positive relationship between the airborne mesophilic bacteria and mold levels on PC1. This suggested a common source of contamination, which is probably related to the number of daily occupants and visitors as well as to the building age (Yang et al., 2009; Junker et al., 2000). In contrast, the mold levels and mesophilic bacteria were found to be reversely related on PC2. It was hence inferred that this might be associated with ventilation performance in investigated buildings (Wu et al., 2007).

In addition to fungal and bacterial transport from the outdoor air to the indoor air, various factors such as number of visitors or extent of indoor traffic are directly related to IAQ in public buildings (Genet et al., 2011). This suggests an inappropriate performance of heating, ventilating, and air-conditioning systems (HVAC) at the site CJT, which might be associated with the elevated number of daily visitors. Therefore, future studies should be extended to not only examine the airborne microbiota loads in public buildings, but also to investigate how the fungal and bacterial transport from the outdoor air may influence the indoor levels of airborne germs, to assess the conditions under which these microorganisms may exert a health hazard in indoor conditions; and to determine how can such risks be overcome.

Conclusion

The present work shows that indoor air quality in heavily trafficked buildings is affected by relatively high fungal and mesophilic bacteria loads. The levels to which these airborne germs are found in public buildings pose little threat to healthy occupants and visitors, but may cause serious infections to those who have more severe impairment of immunity. Improving ventilation performance may be a viable solution to overcome this risk.

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