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Assessment of microbiological indoor air quality in an Italian office building equipped with an HVAC system

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Abstract The purpose of this study was to evaluate the level and composition of bacteria and fungi in the indoor air of an Italian office building equipped with a heating, ventilation and air conditioning (HVAC) system. Airborne bacteria and fungi were collected in three open-space offices during different seasons. The microbial levels in the outdoor air, supply air diffusers, fan coil air flow and air treatment unit humidification water tank were used to evaluate the influence of the HVAC system on indoor air quality (IAQ). A medium–low level of bacterial contamination (50–500 CFU/m^3) was found in indoor air. *Staphylococcus* and *Micrococcus* were the most commonly found genera, probably due to human presence. A high fungal concentration was measured due to a flood that occurred during the winter. The indoor seasonal distribution of fungal genera was related to the fungal outdoor distribution. Significant seasonal and daily variation in airborne microorganisms was found, underlining a relationship with the frequency of HVAC system switching on/off. The results of this monitoring highlight the role of the HVAC system on IAQ and could be useful to better characterise bacterial and fungal population in the indoor air of office buildings.

Keywords Bacteria · Fungi · Indoor air · HVAC system · Office building

Introduction

Indoor air quality (IAQ) is an increasingly important issue for occupational and public health (Clarke and Nikkel 1995; Reynolds et al. 2001). Health effects related to IAQ have increased, perhaps due to some energy-saving measures provided for buildings such as tight sealing (Jones 1999), air recirculation and the introduction of heating ventilation air conditioning (HVAC) systems (Nathanson 1995). In addition, an ageing population, an increasing number of sensitive individuals and a tendency to spend more time indoors (90% of life time) further worsen this problem (Tringe et al. 2008). Although discussions

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about indoor air contamination frequently concentrate on chemical pollutants, the health effects of inhaled biological particles should not be overlooked, as a large variety of biological material is present in indoor environment (Montanaro 1997).

Bioaerosols are airborne particles that are either living (e.g. bacteria, fungi) or originate from living organisms that are ubiquitous, highly variable and complex and are natural or man-made in origin. The sampling and analysis of airborne microorganisms in indoor air has received attention in recent years (Kim and Kim 2007; Huttunen et al. 2008; Stanley et al. 2008). Bioaerosols contribute to about 5–34% of indoor air pollution (Srikanth et al. 2008). The source of bioaerosols in indoor air include furnishing and building materials, microbiological contamination within the walls, and ceiling and floor cavities. Another significant source of airborne bacteria in the indoor environment is the occupants (Loftness et al. 2007; Stanley et al. 2008). Many indoor bioaerosols originate outdoors through the opening of doors and windows or through cracks in the building envelope. However, one of the most important factors affecting IAQ is how the building is heated, ventilated and air conditioned (Seppänen and Fisk 2002), considering that in many cases, particularly in office buildings, these functions are integrated into an HVAC system.

Although HVAC systems can help remove and/or dilute more than 80% of aerosols from the outdoors, they can also provide favourable breeding grounds for bioaerosols to colonise (Law et al. 2001). A microbiological growth may occur in an HVAC system equipped with low-efficiency filters, humidifiers that use water recycling or in areas in which water condensation remains stagnant and large recirculation of the air is present (Wu et al. 2005; Huang et al. 2008). Microorganisms can thus spread in the indoor air by the HVAC system and be inhaled by the people working or living in buildings (Parat et al. 1997; Mendell et al. 2008).

Numerous studies published during the past 10–15 years have produced rather solid scientific evidence that indoor aerosol particles, especially in the respirable fraction, are associated with health effects. Viable bioaerosol particles, including viruses, bacteria and fungi, have been associated with respiratory allergies and asthma and have been linked to the airborne transmission of various infections known as building-related illnesses (e.g. Legionnaires' disease, aspergillosis; Stetzenbach 1997; Huang et al. 2008). Airborne bacteria and fungi can also be the cause of non-specific diseases such as sick building syndrome (SBS). Numerous scientific studies have documented that SBS is surprisingly common even in buildings without widespread health complaints (Mendell et al. 2008). Moreover, epidemiological investigations showed that SBS and hypersensitivity diseases (humidifier fever or asthma) are often associated with high airborne microbial concentration exposure (ACGIH 1999; Shoemaker and House 2006).

To contribute to the knowledge on IAQ, this study evaluates the level and composition of bacterial and fungal contamination of indoor air in an Italian office building equipped with an HVAC system during different seasons. The influence of the HVAC characteristics and human activities on IAQ was also investigated.

Materials and methods

Description of the site

The study was carried out in an office building (35 years old) with a central HVAC system and open-space offices located in a busy and suburban zone of Turin (Piedmont, Italy). There were two or three occupants per office, computers and printers. The HVAC system was composed of the following: an intake for outdoor air located on the roof of the building, vent ducts at the ceiling for air removal, and a duct bringing outdoor air to the air treatment unit (ATU, 4 years old). In the ATU, fresh (50% minimum) and recycled air were mixed and filtered (85% removal for $\geq$3 μm particles prefilter according to ASHRAE 52–76 with a bag-type filter), heated or cooled, dehumidified or humidified (air humidification tank) and distributed through a duct network to vent ducts placed in the false ceiling. Heating and cooling devices (fan coil units) were distributed in open spaces to heat or cool indoor recycled air. The windows were locked, and building occupants could only

regulate the fan coil units by switching them on or off.

Sampling

A diagram of the sampling points is shown in Fig. 1. Three open-space offices (A, B and C) were selected to follow the air duct distribution from the fresh air intake to the end. Air was sampled for microbiological analysis in the centre of each open-space office, at 1.5 m from the floor (at the workstation level) and was collected twice a day (at 9.00 A.M. and 4.30 P.M.), three times a week (Monday, Wednesday and Friday) and 1 week during different seasons (winter, spring, summer). Since an unusual flood occurred during the winter, another sampling was repeated during the following winter (winter2).

To evaluate the HVAC influence on indoor microbiological contamination, additional evaluations were performed once a week (on Wednesday) for each season. In particular, the bacterial and fungal counts at the intake point of the ATU system (Fig. 1) and at the level of the two ventilation shafts in the indoor air (the first and the last ventilation shafts after the ATU system) were analysed. Microbiological analyses were also performed on the ATU humidification water tank. The air flow of the fan coil units was also sampled in the same offices in which IAQ was monitored to evaluate its influence on air contamination. A total of 240 air samples were collected for bacterial and fungal measurements: (three parameters: bacteria at 22°C, bacteria at 37°C and fungi) × (three offices: A, B and C) × (two daily samplings: A.M./P.M.) × (three times a week: Monday, Wednesday and Friday) × (four seasons: winter, spring, summer, winter2) + [(one outdoor sample + two ventilation shafts + three fan coil units) × (four seasons: winter, spring, summer, winter2)].

Airborne bacteria and fungi were collected using a calibrated impactor sampler (SAS Super 180™, International PBI, Milan) at an air flow rate of 180 L/min (sampling time 1.10 and 1.20 min for fungal and bacterial samplings, respectively). Three plates per each sampling point were taken to evaluate the different microbiological parameters and quality assurance/quality control aspect. Field blank plates for quality control (a minimum of one for each medium) accompanied the sampler during the transport and air monitoring. These field blanks were processed and analysed exactly as the other plates.

Microbiological analyses

Total bacterial counts were evaluated on tryptic case soy agar supplemented with cycloheximide (100 μg/L), and fungal counts were performed on Rose-Bengal Agar with chloramphenicol (100 μg/L). Petri dishes were incubated for 24 h at 37°C and 48h at 22°C for bacterial counts and 4–8 days at 24°C for fungal counts. The mean value of the triplicate samples was calculated, and results were expressed in colony forming

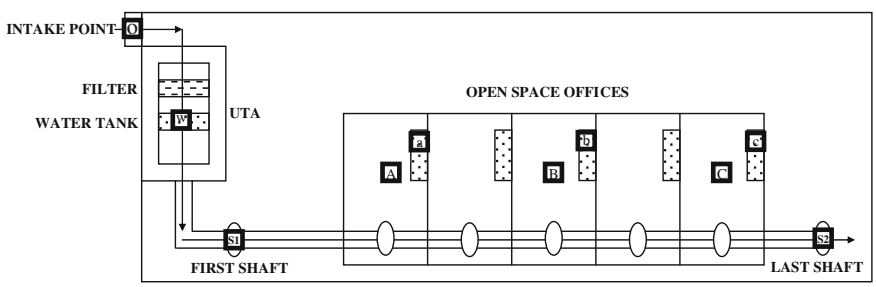

Fig. 1 Diagram of the sampling points in the office building

O: outdoor air;
S_1: first ventilation shaft;
S_2: last ventilation shaft;
W: humidification water tank;
A,B,C: offices;
a,b,c: fan coil units.

units per cubic meter (CFU/m^3), corrected based on the sampler's manufacturer instructions. The consistency of triplicate counts was evaluated using a χ^2 index to confirm the suitability of the mean value of the three measurements. The most widespread bacterial and fungal colonies for each sampling were selected and isolated from indoor and outdoor air samples on the basis of their morphology.

Water samples collected in the air humidification tank of the ATU were analysed for *Legionella* spp. (ISO 1998) and total bacterial counts (22°C and 37°C counts; EN ISO 1999).

Microbial identification

Bacterial identifications by metabolic fingerprinting analysis were performed using the Biolog® system (Biolog® Inc., Hayward, CA, USA), originally created for soil and water microorganisms identification (Avidano et al. 2005). This method tests the ability of a microorganism to utilise or oxidise different carbon sources. Tetrazolium violet is used as a redox dye. Briefly, bacteria were grown on Biolog Universal Growth agar at 37°C for 24 h or at 22°C for 48 h. Colonies were suspended in a 0.4% saline solution and the inoculum density adjusted to the specified turbidity range. The bacterial suspensions were inoculated in a Biolog GP Microplate and were incubated at 37°C or 22°C then manually read after 24 h. The pattern of purple wells was analysed by Biolog® Microlog 2 software.

Fungal genera were identified by colony characteristics and microscopic examination based on their micro- and macro-morphological characteristics using standard taxonomic keys (Von Arx 1981).

Statistical analyses

SYSTAT software for Windows, version 8.0, was used for statistical analysis. Analysis of variance (ANOVA) was performed on bacteria (22°C and 37°C counts) and fungi indoor concentrations using season, day of the working week, and morning or afternoon as main parameters. Pearson's correlation coefficient was used to study the relationship among the microbial parameters analysed.

Results

Microbiological indoor air quality

Table 1 summarises the values of bacterial and fungal indoor contamination during seasonal samplings. For each season, bacterial and fungal counts detected in the three offices (A, B and C) on Monday, Wednesday and Friday were pooled and reported as a weekly mean with minimum and maximum values. The counts observed were compared with categories of contamination indicated in the guidelines of the Commission of European Communities (CEC 1993, Table 2).

The first winter sampling revealed unusually high indoor fungal concentrations. During this sampling, high fungal contamination was found in all offices and a value >2,000 CFU/m^3 was observed in office C (Table 1). This finding is

Table 1 Indoor air bacterial (22°C and 37°C) and fungal concentrations (CFU/m^3) during seasonal samplings

Parameter (CFU/m^3)		Winter ($n = 18$)[a]			Spring ($n = 18$)[a]			Summer ($n = 18$)[a]			Winter2 ($n = 18$)[a]		
		Mean[b]	Min	Max	Mean[b]	Min	Max	Mean[b]	Min	Max	Mean[b]	Min	Max
Bacteria 37°C	m	102	32	248	97	36	256	198	120	368	253	116	496
	a	76	44	128	65	32	96	136	68	324	158	84	268
Bacteria 22°C	m	72	32	200	120	56	320	176	112	240	271	128	500
	a	28	4	56	129	40	308	134	52	368	160	72	304
Fungi 24°C	m	514	50	1,528	321	55	465	44	25	90	49	10	100
	a	872	30	2,315	43	25	100	44	10	75	39	10	70

m morning, *a* afternoon, *Min* minimum value, *Max* maximum value

[a]Number of samples analysed for each parameter

[b]Weekly mean of microbiological counts recovered in the three offices (A, B, and C) on Monday, Wednesday, and Friday

Table 2 Categories of CFU/m^3 (mixed population of bacteria and of fungi) for non-industrial indoor environments (CEC 1993)

Category	Bacteria	Fungi
Very low	<50	<25
Low	<100	<100
Intermediate	<500	<500
High	<2,000	<2,000
Very high	>2,000	>2,000

These categories are based on the range of values obtained in indoor environments and not on a health risk evaluation

probably related to a flood that occurred in Turin near the investigated building during sampling. Massive mould growth was also reported in other studies on flood-damaged homes (Riggs et al. 2008; Rao et al. 2007). For this reason, another sampling was repeated the following winter (winter2).

In the other seasonal samplings (spring, summer and winter2), the fungal concentration decreased, and values lower than 500 CFU/m^3 (medium–low contamination levels in spring) and 100 CFU/m^3 (very low–low contamination levels in summer and winter2) were observed. The low contamination reported in winter2 confirms that the high fungal levels encountered in the first winter sampling do not represent typical winter contamination and that they had been influenced by the flood (Riggs et al. 2008; Rao et al. 2007).

Bacterial concentrations (37°C) ranged between 32 and 496 CFU/m^3 (mean 136 CFU/m^3), with values lower than 500 CFU/m^3 (medium–low contamination levels). A similar trend was observed for bacteria at 22°C, showing a mean concentration of 136 CFU/m^3 (range 4–500 CFU/m^3).

The ANOVA test performed on total raw data (excluding the first winter sampling) revealed significant differences in bacterial (22°C and 37°C) and fungal indoor contamination related to the season (Table 3). The bacterial counts (22°C and 37°C) increased from spring to summer and winter2, while the fungal concentration increased in the spring. Similar trends were also observed in other studies (Sen and Asan 2009). Furthermore, a significant difference in daily bacterial (22°C and 37°C) and fungal indoor contamination was detected, showing a trend with higher contamination levels in the morning than in the afternoon (Table 3). A significant work week trend of bacterial indoor contamination (22°C) of Friday > Wednesday > Monday was revealed by the ANOVA.

No significant relationship among bacterial concentrations at 22°C and 37°C and fungal counts was revealed by the Pearson's correlation coefficient.

HVAC system

Air treatment unit

To study the influence of the ATU system on IAQ, microbial contamination of outdoor and indoor air sampled near supply air diffusers was evaluated (Table 4). Fungal and bacterial (22°C) levels from outdoor air increased from winter to summer, except for the fungal air concentration measured during the flood, which was unusually high. As reported by other authors (Pastuszka et al. 2000; Shelton et al. 2002; Lee et al. 2006), seasonal high temperatures and humidity can increase microbiological activity, leading to an increase of bacterial and fungal counts.

In the first winter sampling, similar fungal counts in outdoor and indoor air diffusers (>3,000 CFU/m^3, SAS upper detection limit) were observed, so it is possible that the ATU could not

Table 3 The analysis of variance on bacterial and fungal counts with respect to season, sampling point (office), day of work week, and sampling hour

	Bacteria 22°C	Bacteria 37°C	Fungi 24°C
Season	$p < 0.001$ (Sp > Su > W2)	$p < 0.001$ (Sp > Su > W2)	$p < 0.001$ (W2 ≈ Su > Sp)
Office	n.s.	n.s.	n.s.
Days of working week	$p < 0.05$ (F > W > M)	n.s.	n.s.
Hour	$p < 0.05$ (m > a)	$p < 0.05$ (m > a)	$p < 0.05$ (m > a)

Values obtained excluded the anomalous winter sample
n.s. not significant, *Sp* spring, *Su* summer, *W2* winter2, *F* Friday, *W* Wednesday, *M* Monday, *m* morning, *a* afternoon

Table 4 Bacterial (22°C and 37°C) and fungal concentration (CFU/m^3) in outdoor air and at the ventilation shafts during seasonal samplings

CFU/m^3		Winter Mean[a] ± SD	Spring Mean[a] ± SD	Summer Mean[a] ± SD	Winter2 Mean[a] ± SD
Bacteria 37°C	O	114 ± 62	96 ± 17	284 ± 86	248 ± 53
Bacteria 22°C	O	189 ± 17	352 ± 57	600 ± 127	308 ± 87
Fungi 24°C	O	>3,268[b]	145 ± 15	695 ± 145	115 ± 8
Bacteria 37°C	S1	20 ± 4	44 ± 5	84 ± 11	132 ± 16
Bacteria 22°C	S1	4 ± 2	144 ± 39	120 ± 30	228 ± 70
Fungi 24°C	S1	>3,268[b]	60 ± 10	70 ± 4	20 ± 8
Bacteria 37°C	S2	48 ± 5	48 ± 20	76 ± 38	76 ± 40
Bacteria 22°C	S2	16 ± 1	104 ± 2	60 ± 28	48 ± 11
Fungi 24°C	S2	>3,268[b]	30 ± 3	50 ± 18	40 ± 23

SD standard deviation, *O* outdoor air collected at the intake point of the ATU system, *S*1 indoor air at the level of the first ventilation shaft after the ATU system, *S*2 indoor air at the level of the last ventilation shaft after the ATU system
[a]Mean value of the three measurements
[b]SAS upper detection limit calculated, according to the manufacturer, considering the maximum number of colonies in the Petri dishes counted with the SAS Super 180

remove the high fungal contamination in the outdoor air. Otherwise, in this sampling, outdoor bacterial values (22°C and 37°C) were lower than fungal counts, and the ATU was able to remove most of it.

During all the other samplings, the microbiological contamination of the air measured at the ATU air diffusers revealed a lower bacterial and fungal concentration than outdoors, with values lower than 500 or 100 CFU/m^3.

Fan coil air

Table 5 summarises bacterial and fungal concentrations in the fan coil unit air flow during seasonal samplings. High fungal contamination (range 500–2,000 CFU/m^3) of the fan coil unit air flow was observed in the first winter sampling when high fungal counts were found in indoor and outdoor air. During other samplings, both bacterial and fungal concentrations were medium–low or sometimes very low (<500 or <50 CFU/m^3), similar levels to that observed in the indoor air.

Indoor–outdoor ratio

The indoor/outdoor ratio (*I*/*O*; Table 6) was calculated using the indoor (mean values obtained in the open-space offices on Wednesday) and outdoor microbial counts obtained during the four samplings. *I*/*O* ratios showed that fungal counts and 22°C bacterial concentrations were always higher outdoors than indoors, in agreement with other studies performed in office buildings (Burge et al. 2000). On the other hand, *I*/*O* values close to 1 for bacterial counts at 37°C indicated higher

Table 5 Bacterial (22°C and 37°C) and fungal concentration (CFU/m^3) in the fan coil unit's air flow during seasonal samplings

Parameter (CFU/m^3)	Winter ($n = 3$)[a]			Spring ($n = 3$)[a]			Summer ($n = 3$)[a]			Winter2 ($n = 3$)[a]		
	Mean[b]	Min	Max	Mean[b]	Min	Max	Mean[b]	Min	Max	Mean[b]	Min	Max
Bacteria 37°C	82	60	113	52	36	80	128	32	200	96	80	112
Bacteria 22°C	36	20	47	71	44	116	95	32	128	180	160	200
Fungi 24°C	935	560	1,465	15	10	20	50	30	65	60	20	100

Min minimum value, *Max* maximum value
[a]Number of samples analysed for each parameter
[b]Mean of microbiological counts recovered at the three fan coils air flow (office A, B, and C) on Wednesday

Table 6 Indoor/outdoor ratios calculated for bacterial and fungal loads in seasonal samplings

Season	I[a]/O[b]		
	Bacteria 22°C	Bacteria 37°C	Fungi 24°C
Winter	0.63	0.82	0.34
Spring	0.39	1.08	0.80
Summer	0.80	1.06	0.38
Winter2	0.70	1.13	0.28

[a]Means of microbiological counts obtained at the level of open-spaces on Wednesday
[b]Microbiological counts obtained at the intake point of the ATU system on Wednesday

bacterial contamination inside the building than outside.

Microbiological analyses of the humidification water tank

Results reported on Table 7 showed that the total bacterial count in the humidification water tank was high (range between 1×10^3 CFU per 100 mL and 1.5×10^5 CFU per 100 mL) with an increasing trend during warmer seasons (spring and summer). *Legionella* spp. was never recovered in the ATU humidification water.

Bacterial identification

The identification of the most widespread bacteria in indoor air found *Micrococcus* (32% of species) and *Staphylococcus* (44%) as the most frequently occurring genera. These results support the general idea that, in indoor air, the main represented bacteria are micrococci and staphylococci (Nevalainen 1989; Maroni et al. 1993). *Micrococcus luteus* and *Staphylococcus haemolyticus* were found in all indoor air samples. Species commonly found on human skin (*Micrococcus lylae*, *M. luteus* and *Staphylococcus saprophyticus*), in the external ear auditory meatus (*Staphylococcus auricularis*) or on the human scalp (*Staphylococcus capitis*) were also found. Some of the identified bacteria (*Staphylococcus epidermidis*, *S. haemolyticus*, *Staphylococcus warneri* and *Staphylococcus hominis*) can be considered to be opportunistic pathogens, while others (*Kurtia gibsonii*, *Kocuria rosea erythromixa* and *Tsukamurella inchonensis*) are typically found in environmental samples. Bacterial species found in the fan coil air flow were similar to those recovered in indoor air. In outdoor air, environmental species such as *Aerococcus viridans* and *T. inchonensis* were mainly encountered.

Considering that to our knowledge, this is the first time that the BIOLOG® system has been used for airborne bacterial identification, this method had a good performance, leading to the good identification of 94.3% of the analysed colonies.

Fungal identification

Figure 2 shows the seasonal distribution of the fungal genera in indoor air. According to other studies, *Penicillium spp.*, *Aspergillus spp.* and *Cladosporium cladosporioides* and *C. sphaerospermum* were the most widespread fungal genera identified in indoor air (Pasanen et al. 1992; Hyvärinen et al. 1993; Nevalainen and Seuri 2005; Sampò and Luppi Mosca 1988, 1989). *Penicillium* was predominant in indoor air during the winter (68%) and spring (60%), while *Cladosporium* was the most widespread genus in summer. This trend was similar to that commonly reported in outdoor air (Jo and Seo 2005). The fungal genera identified (*Penicillium*, *Aspergillus* and *Cladosporium*) in indoor air are recognised as possible causes of respiratory allergies (Jones 1999), and *Penicillium* species can be associated with sick building syndrome (Schwab and Straus 2004).

Table 7 Microbiological contamination of humidification water tank

	Winter	Spring	Summer	Winter2
Bacteria at 37°C (CFU/100 mL)	1,900	29,200	43,000	1,800
Bacteria at 22°C (CFU/100 mL)	1,100	150,000	136,000	15,600
Legionella spp. (CFU/L)	<100[a]	<100[a]	<100[a]	<100[a]

[a]<100 CFU/L: detection limit for *Legionella* spp. analysis

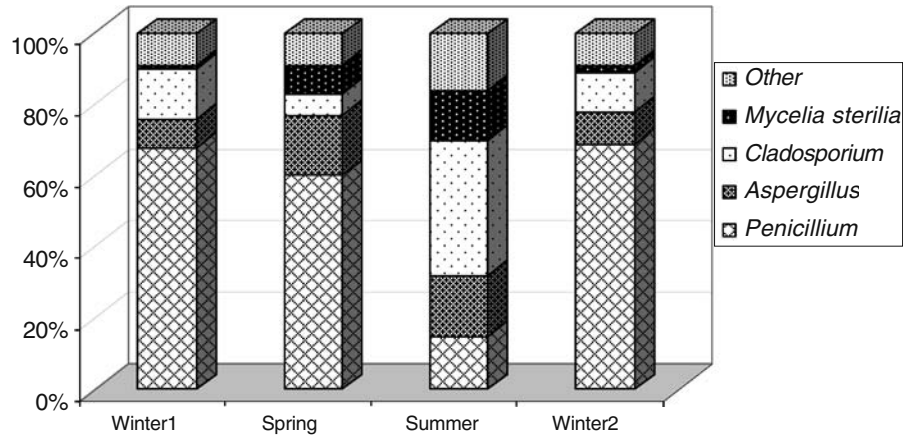

Fig. 2 Distribution of fungal genera in indoor air during different seasons

Discussion

This study evaluated the number and composition of bacteria and fungi in the indoor air of an Italian office building equipped with an HVAC system. It was not performed in response to occupant complaints but rather to describe the building's air quality over time. As represented by our data, bacterial and fungal concentrations in all offices ranged from 50 to 500 CFU/m^3, indicating a medium–low contamination level (CEC 1993). The values obtained were in the range reported in other studies of non-complaint office buildings around the world (Parat et al. 1997; Law et al. 2001; Sessa et al. 2002; Shelton et al. 2002; Tsai and Macher 2005). On the other hand, during the first winter sampling, a high fungal contamination was recorded (range between 500 and 2,000 CFU/m^3) in indoor air. This result is probably related to the flood that occurred during that period, leading to water stagnation and environmental conditions (e.g. relative humidity and nutrients) favouring fungal growth. No significant difference in the microbial air contamination was found among the offices, probably because the three offices considered were located in an open space without any particular source of microbial contamination. This homogeneous distribution of bacterial and fungal counts was also observed in other studies (Parat et al. 1997).

Considering the bacterial species identified in indoor air, the predominance of *Micrococcus* and *Staphylococcus* genera could be due to the human presence. This finding is supported by the indoor/outdoor ratio close to 1 for bacteria at 37°C ($0.82 \leq I/O \leq 1.13$). As observed in other studies (Pastuszka et al. 2000), the high bacterial count within the building compared to that observed outdoor could be associated with various internal sources, including human activities. Otherwise, the fungal I/O ratio <1 ($0.28 \leq I/O \leq 0.8$) indicated the absence of an indoor air contamination source. Moreover, as observed by Burge et al. (2000) and Shelton et al. (2002), the seasonal distribution of fungal genera in indoor air confirmed the influence of outdoor air. The data analysis revealed the absence of any relationship between bacterial and fungal counts, confirming their origin from different sources.

Although some bacterial species encountered in indoor air can be considered to be opportunistic pathogens (*S. haemolyticus*, *S. epidermidis* and *S. hominis*), the heterogeneity of the bacterial species and the low counts recorded do not suggest any particular risk for occupant health. In contrast, the high fungal contamination and the presence of potentially allergenic genera (*Penicillium*) observed in the first winter sampling (related to the flood) point out a possible health risk in that situation.

Important information about influence of the HVAC characteristics (e.g. ATU, fan coil, water) on IAQ can be obtained from the detailed analysis of bacterial and fungal counts. In particular, the significant daily trend of indoor air bacterial (22°C and 37°C) and fungal contamination could be associated with air movement caused by the daily HVAC system being switched on in the morning

and switched off at the end of the working day. This trend suggests that bacteria and fungi could proliferate in the ATU filter and vent ducts when the HVAC system is switched off. Then, when the HVAC system is switched on, the microorganisms could spread in the air. The emission of bacteria and fungi due to incubation during the nighttime HVAC shutdown was also observed in other studies (Law et al. 2001).

The significant seasonal trend of microbial counts observed in indoor air, which differed from outdoor air, was probably due to many factors such as internal air circulation and the intermittent use of HVAC system, which varied according to the season. Therefore, as observed by other authors (Parat et al. 1997; Tsai and Macher 2005), indoor microbial counts do not seem to be related to outdoor levels in the presence of low microbial contamination. The results of the ATU investigation demonstrated the good efficiency of microbial contamination removal of the unit. Except for the first winter sampling, in normal climatic conditions the ATU seems to have removed air microbial contamination.

A relevant role in air movement might be attributed to building occupants' personal choice to switch on or off the fan coil units. The analysis of the fan coil unit air flow revealed microbial contamination levels lower than the indoor air. Moreover, bacterial species found in the fan coil unit air flow were similar to those recovered in the indoor air. These results suggest that fan coil units determined air movement, but in this case, they were not directly involved in indoor air contamination.

In regards to the water investigation, high bacteria in the humidification water tank was recorded, underlining a microbial proliferation, especially during warmer seasons.

In conclusion, this study can help better characterise bacterial and fungal populations in the indoor air of office building, highlighting the role of the HVAC system on IAQ. The results obtained suggest that the uninterrupted functioning of the HVAC system may be recommended, with the possible application of an energy-saving device. Moreover, a programmed and periodical cleaning operation and maintenance activities of the HVAC system should be organised as a preventive measure. It was also speculated that great care should be taken during the cleaning operation of the water tank in the ATU system. Although in the investigated building there was no particular risk observed, except for the unusual flood, it is important to note the usefulness of an IAQ preventive evaluation to avoid occupant complaints or health effects. In fact, although the preventive investigation may be initially more expensive and time consuming, it may prove to be useful by preventing building environmental problems whose solution could be very difficult and need greater investment.

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References

ACGIH (1999). *Bioaerosol: Assessment and control* (1st ed.). Cincinnati, OH: American Conference of Governmental Industrial Hygienists.

Avidano, L., Gamalero, E., Cossa, G. P., & Carraro, E. (2005). Characterisation of soil health in an Italian polluted site by using microorganisms as bioindicators. *Applied Soil Ecology, 30*, 21–33. doi:10.1016/j.apsoil.2005.01.003.

Burge, H. A., Pierson, D. L., Groves, T. O., Strawn, K. F., & Mishra, S. K. (2000). Dynamics of airborne fungal populations in a large office building. *Current Microbiology, 40*, 10–16. doi:10.1007/s002849910003.

CEC (1993). *Indoor air quality & its impact on man. Biological particles in indoor environments.* Commission of European Communities. Report 12. Cost Project 613. EUR. 14988 EN.

Clarke, D., & Nikkel, D. (1995). *Office air: A worker's guide to air quality in offices, schools, and hospitals*. Report of the Federal–Provincial Advisory Committee on Environmental and Occupational Health, Canada. 93-EHD-174.

EN ISO (1999). *Water quality—Enumeration of culturable micro-organism—colony count by inoculation in a nutrient agar culture medium*. International Organization for Standardization (EN ISO Standard 6222–1999).

Huang, R., Agranovski, I., Pyankov, O., & Grinshpun, S. (2008). Removal of viable bioaerosol particles with a low-efficiency HVAC filter enhanced by continuous emission of unipolar air ions. *Indoor Air, 18*, 106–112. doi:10.1111/j.1600-0668.2007.00512.x.

Huttunen, K., Rintala, H., Hirvonen, M. R., Vepsäläinen, A., Hyvärinen, A., Meklin, T., et al. (2008). Indoor air particles and bioaerosols before and after renovation of moisture-damaged buildings:the effect on biological

activity and microbial flora. *Environmental Research, 107*, 291–298. doi:10.1016/j.envres.2008.02.008.

Hyvärinen, A., Reponen, T., Husman, T., Ruuskanen, J., & Nevalainen, A. (1993). Characterizing mold problem buildings: Concentrations and flora of viable fungi. *Indoor Air, 3*, 337–343. doi:10.1111/j.1600-0668.1993.00017.x.

ISO (1998). *Water quality-detection and enumeration of Legionella*. International Organization for Standardization (ISO Standard 11731–1998).

Jo, W. K., & Seo, Y. J. (2005). Indoor and outdoor bioaerosol levels at recreation facilities, elementary schools, and homes. *Chemosphere, 61*, 1570–1579. doi:10.1016/j.chemosphere.2005.04.103.

Jones, A. P. (1999). Indoor air quality and health. *Atmospheric Environment, 33*, 4535–4564. doi:10.1016/S1352-2310(99)00272-1.

Kim, K. Y., & Kim, C. N. (2007). Airborne microbiological characteristics in public buildings of Korea. *Building and Environment, 42*, 2188–2196. doi:10.1016/j.buildenv.2006.04.013.

Law, A. K. Y., Chau, C. K., & Chan, G. Y. S. (2001). Characteristics of bioaerosol profile in office buildings in Hong Kong. *Building and Environment, 36*, 527–541. doi:10.1016/S0360-1323(00)00020-2.

Lee, T., Grinshpun, S. A., Martuzevicious, D., Adhikari, A., Crawford, C. M., Luo, J., et al. (2006). Relationship between indoor and outdoor bioaerosols collected with a button inhalable aerosol sampler in urban homes. *Indoor Air, 16*, 37–47. doi:10.1111/j.1600-0668.2005.00396.x.

Loftness, V., Hakkinen, B., Adan, O., & Nevalainen, A. (2007). Elements that contribute to healthy building design. *Environmental Health Perspectives, 115*, 965–970.

Maroni, M., Bersani, M., Cavallo, D., Anversa, A., & Alcini, D. (1993). Microbial contamination in buildings: Comparison between seasons and ventilation systems. In O. Seppanen (Ed.), *Proceedings of indoor air'93, Helsinki, international conference on indoor air, quality and climate* (Vol. 4, pp. 137–142).

Mendell, M. J., Lei-Gomez, Q., Mirer, A. G., Seppänen, O., & Brunner, G. (2008). Risk factors in heating, ventilating, and air-conditioning systems for occupant symptoms in US office buildings: The US EPA BASE study. *Indoor Air, 18*, 301–316. doi:10.1111/j.1600-0668.2008.00531.x.

Montanaro, A. (1997). Indoor allergens: Description and assessment of health risks. In E. J. Bardana, & A. Montanaro (Eds.), *Indoor air pollution and health*. New York: Marcel Dekker.

Nathanson, T. (1995). *Indoor air quality in office buildings: A technical guide*. Report of the Federal–Provincial Advisory Committee on Environmental and Occupational Health, Canada. 93-EHD-166.

Nevalainen, A. (1989). *Bacterial aerosols in indoor air*. Ph.D. diss, University of Kuopio, Finland.

Nevalainen, A., & Seuri, M. (2005). Of microbes and men. *Indoor Air, 15*, 58–64. doi:10.1111/j.1600-0668.2005.00344.x.

Parat, S., Perdrix, A., Fricker-Hidalgo, H., Saude, I., Grilloy, R., & Baconnier, P. (1997). Multivariate analysis comparing microbial air content of an air-conditioned building and a naturally ventilated building over one year. *Atmospheric Environment, 31*, 441–449. doi:10.1016/S1352-2310(96)00212-9.

Pasanen, A., Niininen, M., Kalliokoski, P., Nevalainen, A., & Jantunen, M. (1992). Airborne *Cladosporium* and other fungi in damp versus reference residences. *Atmospheric Environment, 26*, 121–124.

Pastuszka, J. S., Paw, U. K. T., Lis, D. O., Wlazlo, A., & Ulfig, K. (2000). Bacterial and fungal aerosol in indoor environment in Upper Silesia, Poland. *Atmospheric Environment, 34*, 3833–3842. doi:10.1016/S1352-2310(99)00527-0.

Rao, C. Y., Riggs, M. A., Chew, G. L., Muilenberg, M. L., Thorne, P. S., Van Sickle, D., et al. (2007). Characterization of airborne molds, endotoxins, and glucans in homes in New Orleans after hurricanes Katrina and Rita. *Applied and Environmental Microbiology, 73*, 1630–1634. doi:10.1128/AEM.01973-06.

Reynolds, S. J., Black, D. W., Borin, S. S., Breuer, G., Burmeister, L. F., Fuortes, L. J., et al. (2001). Indoor environmental quality in six commercial office buildings in the midwest United States. *Applied Occupational and Environmental Hygiene, 16*, 1065–1077. doi:10.1080/104732201753214170.

Riggs, M. A., Rao, C. Y., Brown, C. M., Van Sickle, D., Cummings, K. J., Dunn, K. H., et al. (2008). Resident cleanup activities, characteristics of flood-damaged homes and airborne microbial concentrations in New Orleans, Louisiana, October 2005. *Environmental Research, 106*, 401–409. doi:10.1016/j.envres.2007.11.004

Sampò, S., & Luppi Mosca, A. M. (1988). Fungi from the walls of a flat in Turin. *Allionia, 28*, 175–184.

Sampò, S., & Luppi Mosca, A. M. (1989). A study of the fungi occurring on 15th century frescoes in Florence, Italy. *International Biodeterioration, 25*, 343–353.

Schwab, C. J., & Strauss, D. C. (2004). The roles of *Penicillium* and *Aspergillus* in sick building syndrome. *Advances in Applied Microbiology, 55*, 215–238.

Sen, B., & Asan, A. (2009). Fungal flora in indoor and outdoor air of different residential houses in Tekirdag City (Turkey): Seasonal distribution and relationship with climatic factors. *Environmental Monitoring and Assessment*. doi:10.1007/s10661-008-0262-1.

Seppänen, O., & Fisk, W. J. (2002). Association of ventilation system type with SBS symptoms in office workers. *Indoor Air, 12*, 98–112. doi:10.1034/j.1600-0668.2002.01111.x.

Sessa, R., Di Pietro, M., Schiavoni, G., Santino, I., Altieri, A., Pinelli, S., et al. (2002). Microbiological indoor air quality in healthy buildings. *The New Microbiologica, 25*, 51–56.

Shelton, B. G., Kirkland, K. H., Flanders, W. D., & Morris, G. K. (2002). Profiles of airborne fungi in buildings and outdoor environments in the United States. *Applied and Environmental Microbiology, 68*, 1743–1753. doi:10.1128/AEM.68.4.1743-1753.2002.

Srikanth, P., Sudharsanam, S., & Steinberg, R. (2008). Bioaerosols in indoor environment: Composition, health effects and analysis. *Indian Journal of Medical Microbiology, 26*, 302–312.

Stetzenbach, L. D. (1997). Introduction to aerobiology. In C. J. Hurst, G. R. Knudsen, M. J. Mc Inerney, & L. D. Stetzenbach (Eds.), *Manual of environmental microbiology*. Washington, DC: ASM Press.

Stanley, N. J., Kuehn, T. H., Kim, S. W., Raynor, P. C., Anantharaman, S., Ramakrishnan, M. A., et al. (2008). Background culturable bacteria aerosol in two large public buildings using HVAC filters as long term, passive, high-volume air samplers. *Journal of Environmental Monitoring, 10*, 474–481. doi:10.1039/b719316e.

Shoemaker, R. C., & House, D. E. (2006). Sick building syndrome (SBS) and exposure to water-damaged buildings: Time series study, clinical trial and mechanisms. *Neurotoxicology and Teratology, 28*, 573–588. doi:10.1016/j.ntt.2006.07.003.

Tringe, S. G., Zhang, T., Liu, X., Yu, Y., Lee, W. H., Yap, J., et al. (2008). The airborne metagenome in an indoor urban environment. *PLoS ONE, 3*, 1–10.

Tsai, F. C., & Macher, J. M. (2005). Concentrations of airborne culturable bacteria in 100 large US office buildings from the BASE study. *Indoor Air, 15*, 71–81. doi:10.1111/j.1600-0668.2005.00346.x.

Von Arx, J. A. (1981). *The genera of fungi sporulating in pure culture*. Lubrecht & Cramer Ltd. Vaduz, Germany.

Wu, P. C., Li, Y. Y., Chiang, C. M., Huang, C. Y., Lee, C. C., Li, F. C., et al. (2005). Changing microbial concentrations are associated with ventilation performance in Taiwan's air-conditioned office buildings. *Indoor Air, 15*, 19–26. doi:10.1111/j.1600-0668.2004.00313.x.