MICROBIAL CONTAMINATION IN BUILDINGS: COMPARISON BETWEEN SEASONS AND VENTILATION SYSTEMS

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ABSTRACT

Airborne microorganism contamination was investigated in naturally and mechanically ventilated buildings. Air was sampled with SAS system and cultured on general media for total count and on media for fungi; data were related to other indoor contaminants and to microclimatic parameters. Comparison of winter versus summer microorganism concentrations was not significant. No major difference of detected species was observed between naturally and mechanically ventilated buildings. In mechanically ventilated buildings significant correlations between microorganisms and relative humidity were found during the wintertime; a negative correlation between bacteria and particulate matter and a positive correlation between bacteria and temperature were found in summertime.

INTRODUCTION

Indoor airborne biological pollutants may cause various diseases. Attention is especially focused on spreading of microorganisms, their toxins and antigenic materials through centralized air conditioning systems (1-4). Little information is available on indoor concentrations of microorganisms, their seasonal variability and relationship with other indoor contaminants (5). The aim of this study was to determine airborne microorganism contamination in naturally ventilated buildings and in buildings with a heating-ventilating-air conditioning (HVAC) system; data were related to airborne particulate matter, total volatile organic compounds (TVOCs) and some microclimatic parameters.

MATERIALS AND METHODS

A total of 17 buildings were investigated: 10 naturally ventilated schools (3 nurseries, 1 kindergarden, 3 primary and 3 secondary schools) and 7 mechanically ventilated office buildings. The main campaign was performed in wintertime; office buildings (offices and HVAC system) were monitored also during the summer. Air samples were collected inside the buildings and outdoors and were analyzed to assess microbiological and chemical pollutants.

Micro-organisms. The SAS impactor (Surface Air System, PBI International, Italy) was used to collect air volumes of 180 l. Before each sampling the instrument heads were washed with ethanol 70% for 5 minutes.

Culture media and conditions were:
- nutritional plate count agar (PCA) for total microbial count; incubation at 30°C or 35°C for 48h;
- Sabouraud agar or Rose-Bengal chloramphenicol agar, specific for determination of fungi; incubation at 30°C or 35°C for 48 h.

The colonies were counted and some of the samples were studied further for the identification of microorganisms with conventional microbiological methods. The number of counted colonies was corrected on the basis of a statistical probability table supplied by the manufacturer and the counting results were expressed as colony forming units per cubic meter (CFU/m^3) of air.

TVOCs. TVOCs were captured on Carbotrap tubes by means of active samplers and analysed by GC/FID (6).

Particulate matter. Particulate matter < 10 μm (PM 10) was collected on membranes by means of active samplers equipped with Lippmann selector and analysed by gravimetric method (6).

Microclimatic parameters. Temperature, relative humidity and air velocity were measured by LSI-ANADATA system.

Statistical analysis was performed using the SPSS package (Statistical Package for Social Science).

RESULTS

Data on winter concentration of airborne bacteria and fungi in naturally ventilated buildings are shown in Table 1. The distribution of bacteria and fungi values were close to lognormal and, therefore, the results are expressed as geometric means. Bacteria concentration in naturally ventilated buildings ranged from 33 to 5020 CFU/m^3, and the values were higher in primary and secondary schools than in nurseries and kinder-gardens. Fungi ranged from 5 to 55 CFU/m^3. No significant differences were observed between fungi concentrations obtained on PCA and on Sabouraud media; a difference ($p < 0.05$) was observed between CFU values obtained in the two groups of schools only on specific medium.

Indoor bacteria concentrations were higher than outdoor and distributed according to occupancy density; no differences were found for indoor and outdoor fungi concentrations. Staphylococcus (present in 85% of the samples), Micrococcus (present in 72% of the samples) and difteroides (present in 70% of the samples) were the most commonly detected Gram pos. bacteria; Staphylococcus aureus was also identified in 10% of the samples. Acinetobacter lwoffi (present in 42% of the samples) and A. calcoaceticus (present in 17% of the samples) were the most commonly identified Gram neg. bacteria (Table 4). Aspergillus and Penicillium were the most common among the identified fungi genera. No significant correlations were found between microorganisms and microclimatic parameters or TVOCs or particulate matter; a weak negative correlation was observed between fungi and TVOCs ($r = -0.40$, $p = 0.01$).

Data on summer and winter concentrations of airborne bacteria and fungi in mechanically ventilated buildings are shown in Tables 2 and 3. Bacteria and fungi values were normally distributed and the results are expressed as arithmetic means. Bacteria concentration in offices ranged from 28 to 611 CFU/m^3 in summer and from 6 to 266 CFU/m^3 winter; the comparison of winter versus summer bacteria concentration was not statistically significant. Fungi ranged from 6 to 100 CFU/m^3 in summer and from 17 to 278 CFU/m^3 in winter; because of a methodological difference (medium and incubation temperature) of fungi concentrations between seasons can not be compared. Fungi concentrations were higher outdoors than indoors and this was explained by lack of important indoor sources. No significant differences were observed between fungi concentrations obtained on PCA and Sabouraud medium or selective Rose-Bengal agar. Staphylococcus, Micrococcus and difteroides were the most common among the detected Gram pos. bacteria; Staphylococcus aureus was not found; Pseudomonas spp, Acinetobacter spp and Flavobacterium spp were the most common identified Gram neg. bacteria (Table 5). Aspergillus and Penicillium were the most common among the identified fungi genera.

In offices significant correlations were found between microorganisms and relative humidity (bacteria $r = 0.55$; fungi $r = 0.59$) during the wintertime. A negative correlation ($r = -0.58$) was found between bacteria and particulate matter below 10 um and a positive correlation ($r = 0.56$) between bacteria and temperature in summertime. No correlation was found between bacteria or fungi concentrations and TVOCs. In both seasons, bacteria CFU/m^3 in the air emitted from the HVAC system into the offices were significantly lower than in the air conditioning core unit and in the office environments.

DISCUSSION AND CONCLUSIONS

The presence of HVAC system has been observed to influence the concentrations of airborne bacteria (lower in mechanically ventilated buildings), while no important effects have been seen on fungi concentrations. No seasonal differences were observed in bacteria concentration in offices. No difference in fungi CFU were observed between PCA and Sabouraud medium or PCA and selective Rose-Bengal medium and the difference observed between fungi concentrations in summer and winter could be due to the different culture methodology. The weak negative correlation observed in schools between TVOCs and fungi could be explained assuming that TVOCs are positively associated with frequency of cleaning (6). The positive correlation in offices between humidity or temperature and bacteria or fungi concentrations could be due to better conditions for growth. The negative correlation in offices between PM 10 and bacteria concentrations could be due either to the efficiency of the sampler (7) or to a disuniform distribution of microorganisms in the different granulometric fractions of the airborne particulate. The results observed upstream and downstream the HVAC systems indicate that air filtration was effective and important sources of microorganisms were not present inside the HVAC systems. Thus our findings suggest that correctly maintained HVAC systems are effective in reducing indoor airborne bacteria concentrations.

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Table 1. Concentrations (CFU/m³) of airborne bacteria and fungi in naturally ventilated buildings (schools).

BUILDINGS		BACTERIA [a] PCA	FUNGI [a] PCA	FUNGI [a] Sabouraud
Nurseries and kindergardens	no.	16	9	13
	GM	243	15	11 *
	GSD	2.79	2.5	1.98
	min-max	33-1489	5-55	5-39
Primary and secondary schools	no.	24	22	23
	GM	419	14	18 *
	GSD	3.3	2.08	1.76
	min-max	39-5020	5-50	5-44
Total schools	no.	40	31	36
	GM	339	14	15
	GSD	3.2	2.18	1.9
	min-max	33-5020	5-55	5-44
Outdoor	no.	9	8	9
	GM	36	20	14
	GSD	2.8	2.2	1.79
	min-max	5-117	5-50	5-33

a Incubation temperature: 35°C
* Student's T-test: $p < 0.05$

Table 2. Concentrations (CFU/m³) of airborne bacteria in HVAC buildings.

Sampling site		SUMMER [a]	WINTER [b]
AIR CONDITIONING UNIT	no.	9	13
	Mean (SD)	83 (61)	109 (92)
	min - max	11 - 183	6 - 317
VENTILATION DUCTS	no.	9 *	17 *
	Mean (SD)	14 (10)	30.5 (25)
	min - max	6 - 33	6 - 83
OFFICES	no.	25	25
	Mean (SD)	173 (170)	113 (83)
	min - max	28 - 611	6 - 266
OUTDOOR	no.	15	11
	Mean (SD)	56 (47)	74 (84)
	min - max	6 - 161	6 - 283

a Incubation temperature: 35°C b Incubation temperature: 30°C
* Student's T-test: summer vs winter $p < 0.05$

Table 3. Concentrations (CFU/m³) of airborne fungi in HVAC buildings.

Sampling site		SUMMER [a] PCA	SUMMER [a] Sabouraud	WINTER [b] PCA	WINTER [b] Rose-Bengal + Chloramphenicol
AIR CONDITIONING UNIT	no.	5	5	14	15
	Mean (SD)	13.5 (11)	19 (10)	56 (45)	59 (56)
	min - max	6 - 33	6 - 28	6 - 139	6 - 211
VENTILATION DUCTS	no.	1	4	7	3
	Mean (SD)	6	6	18 (22)	8 (3)
	min - max		6 - 6	6 - 67	6 - 11
OFFICES	no.	7	8	17	16
	Mean (SD)	10 (4)	15.5 (15)	23 (27)	27 (29)
	min - max	6 - 17	6 - 45	6 - 94	6 - 83
OUTDOOR	no.	12	12	11	11
	Mean (SD)	34 (25)	30 (26)	84 (83)	97 (83)
	min - max	6 - 83	6 - 100	11 - 250	17 - 278

a Incubation temperature: 35°C b Incubation temperature: 30°C

Table 4. Frequencies of detection of bacteria in air samples of schools.

IDENTIFIED BACTERIA (from PCA)	Nurseries and kindergardens		Primary and secondary schools	
	Frequency of detection (%)	Geom. mean (CFU)	Frequency of detection (%)	Geom. mean (CFU)
STAFILOCOCCUS spp	87	13	83	14
MICROCOCCUS spp	69	8	75	6
DIFTEROIDES	69	7	71	12
STREPTOCOCCUS ANAEMOL.	19	11	25	11
STREPTOCOCCUS ALFA-EMOL.	12	2.5	12	19
STAFILOCOCCUS AUREUS	12	3.3	8	3
OTHER GRAM POS. COCCI	0	n.d.	4	115
GRAM POS. RODS (non-spore forming)	25	7	62	3
ACINETOBACTER LWOFFI	37	4	46	13
ACINETOBACTER CALCOACETICUS	25	2	8	1
PSEUDOMONAS spp	12	4	25	2.6
FLAVOBACTERIUM spp	6	2	12	1.5
GRAM NEG. RODS (other than Enterobacteriaceae)	31	4	29	4

Table 5. Frequencies of detection of bacteria in air samples of offices.

IDENTIFIED BACTERIA (from PCA)	Summer		Winter	
	Frequency of detection (%)	Mean (CFU)	Frequency of detection (%)	Mean (CFU)
STAFILOCOCCUS spp	96	22	36	6
GRAM POS. RODS (non-spore forming)	68	13	12	13
MICROCOCCUS spp	40	3	12	2
DIFTEROIDES	24	20	12	7
PSEUDOMONAS spp	16	9	12	2
ACINETOBACTER spp	4	1	8	2
FLAVOBACTERIUM spp	0	-	8	2
GRAM NEG. RODS (other than Enterobacteriaceae)	8	3	0	-