Indoor airborne microbial load in a Spanish university (University of Murcia, Spain).

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Resumen

Carga microbiológica del aire interior en una universidad española (Universidad de Murcia, España)

El control del aire es parte de la garantía de calidad de ambientes interiores. Se ha realizado un análisis microbiológico del aire en zonas de la Facultad de Biología de la Universidad de Murcia (España) determinando niveles y tipos de microorganismos en suspensión. Las muestras se recogieron por un método de impacto, analizando viables (bacterias y hongos) en presencia o ausencia de personal para valorar la contaminación que introduce la actividad humana. Además, la comparación de la densidad microbiana del interior con la del aire exterior indicó una concentración bacteriana más elevada en el interior. La mayoría de bacterias identificadas fueron cocos gram-positivos de los géneros *Micrococcus*, *Staphylococcus* y *Streptococcus*. Entre los hongos, el aislado más frecuente fue *Cladosporium*. Los resultados revelan una concentración microbiana aérea que corresponde a un nivel de contaminación bajo-intermedio según las pautas establecidas por la Comisión de las Comunidades Europeas en 1993.

Palabras clave: Carga microbiana, Bacterias y hongos en aire interior, Calidad del aire.

Abstract

The control of indoor air is related to the guarantee of quality for internal environments. The air of representative areas within the Faculty of Biology of the University of Murcia (Spain) was analyzed to determine the level of microbial types most frequently found in suspension. Samples were collected by an impaction method and viable counts (bacteria and fungi) determined both in absence or presence of human activities to evaluate the human contribution to the microbial contamination. Also, a comparison between indoor and outdoor microbial population densities showed that the bacterial concentration was higher inside than in the outside. Most bacteria identified were gram-positive cocci belonging to *Micrococcus*, *Staphylococcus* and *Streptococcus* species, whereas *Cladosporium* was the predominant genus isolated among fungi. The microbial concentration of indoor air was within a range which indicates a low-intermediate level of contamination according to the guidelines established in 1993 by the European Community Commission.

Key words: Microbial aerial load, Indoor bacteria and fungi, Air quality.

Introduction

Although the air does not represent a true ecosystem or aeroplancton whereby the microorganisms can grow and reproduce, it does contains microbial forms maintained in suspension coming from the soil, water, plants or animals, including men (Atlas & Bartha 2002). Air movements favor the maintenance of microorganisms in the aerial media while their deposition is barely affected by gravity due to their small size. Factors as temperature, humidity, light and nutrient availability are determinants of microbial survival and abundance. Although pathogenic species are rather scarce in the air, some relevant microorganisms travel by aerial transmission and are involved in serious processes causing pneumonia and other diseases. Aerial fungi are much more important than bacteria as agents for allergic diseases. Many fungal species of *Penicillium*, *Aspergillus*, *Alternaria* and *Cladosporium* have been shown to trigger rhinitis, asthma and dermatitis (Burge 1985).

Respiration by men is not limited to open atmospheric media. The daily intake of air by breathing in closed environments greatly outnumbers the ingestion of other elements like water or nutrients (Cardona, 2003). These and other arguments justify the recent concern about the microbiological quality of the indoor air. The term indoor air usually applies to the air of non-industrial interior environments, like colleges, hospitals, offices, restaurants, homes and similar partially closed settings. The indoor air quality (IAQ) began to be taken into consideration during the past 60´s when the problems on biological contamination came into the focus of attention for earlier environmental researchers (Pelczar & Reid 1966). Since air is an important vehicle for the dissemination of infectious agents and allergic components developing potential undesirable effects on human beings, the control of the microbial charge became an important key to define the environmental quality of ambient media surrounding wide human populations which are largely exposed to indoor air during their daily activities.

The IAQ of a particular building depends on several parameters, such as the quality of the outdoor air, the design of both the ventilation and air conditioning systems (including their operative and maintenance conditions), the compartments in which are divided, as well as the endogenous sources of contamination and their magnitude (Vargas & Gallego 2005). The most frequent defects in quality derive from inadequate ventilation, pollution generated inside the building and contamination from exogenous origin. When more than 20% of the occupants of a building complain about the quality of the air or show clear symptoms of drowsiness, fatigue, nausea, cough, asthma or related respiratory disorders, the phenomenon is known as the *"sick building syndrome"*. In general, predominant bacteria in indoor air are gram-positive and usually not dangerous for human health but, because they often derive from the skin and the respiratory tract of occupants, high viable counts are used as markers of crowded conditions and poor ventilation.

Recently, we published data on the microbiological quality of the outdoor air in open urban areas of the city of Murcia, Spain (Soto et al. 2009). In this work we complement those results by studying the microbial charge of the indoor air in a university institution of the same city. The building of the Faculty of Biology of the University of Murcia contains classrooms, departmental services, research laboratories, cafeteria, library and administration offices. The type of learning activities carried out in this institution includes both practical and theoretical lectures, so that students and professors spend most of the working day inside such building. The microbial density was analyzed in several areas in the presence and absence of users to assess for the contamination introduced by the human activity and to determine the microbiological status of this environment which had not been studied previously.

Materials and methods

Sampling areas and technique

To check for the indoor bacterial and fungal microflora in the air, ten representative sampling points were selected according to the different intensity of human activities inside the analyzed building. The main characteristics of the selected points are shown in Table 1. The total number of collected samples amounted to 414. Three additional points located outside the building were established as reference controls for outdoor air.

Samples were collected in working days throughout March 2009 by using a portable

Point	Place	Characteristics
1	Classroom 0.5	Lectures on elective subjects with relative frequency of students low
2	Classroom 0.6	Lectures in matters of low enrolment
3	Classroom 0.7	Lectures with high number of attending students
4	Aulario I Lecture room	Lectures with high number of attending students
5	Hall	Main building entrance and exit
6	Cafeteria	Maintained level of users, food and beverages supplies
7	Library	Continuous passage area
8	Toilets	Continuous passage area
9	Reception	Closed area into the hall with intermittent passage
10	Office area	Administrative zone with intermittent passage

Tabla 1. Puntos de muestreo y principales características.

Table 1. Sampling areas and main characteristics.

MAS−100® air sampler (Merck) as described previously (Meier & Zingre 2000, Soto et al. 2009). For each point duplicated samples were obtained for microbial counting twice a day, either at 8 am (absence of users in the facilities) or at 1 pm (human presence). Microbial concentration was expressed as colony forming units per m³ of air (CFU/m^3).

Microbial growth and analysis

Commercial culture media were supplied by VWR Internacional Eurolab, S.L. Non-selective nutrient agar was used for general isolation and total bacterial counting. Agar-MacConkey was employed as selective medium to isolate and identify enterobacteria of the coliform group. Growth and isolation of demanding bacteria, as well as determination of haemolytic activity, was assayed on Columbia blood-agar medium. In addition, the selective and differential medium Chapman Stone was used for isolation and identification of gelatinase producing staphylococci. Estimation of airborne fungi was carried out by growth on Sabouraud-agar medium supplemented with chloramphenicol. All culture media were sterilized by autoclaving and spread into sterilized Petri dishes which were then coupled to the air sampler as needed. After the sampling procedure, the plates were incubated for 48 h at 37ºC in the case of bacterial analysis and for 72 h at 28ºC in the case of fungal determination. Since in all cases 100 liters of air were sampled, the number of viable counts obtained in each plate was multiplied by 10 to reflect the microbial content (as CFU) in 1 m³ of captured air.

Microbial identification

In addition to microscope observations, bacterial identification included routine tests such as Gram, Ziehl-Neelsen and Wirtz staining, slant cultures on Kligler iron-agar, oxidase reaction with Kovac`s reagent, catalase activity, and estimation of haemolytic and gelatinase activities. Moreover, isolated colonies of bacteria were also cultured on Hugh-Leifson differential medium to assess for fermentative or oxidative properties and motility. In some cases, bacterial colonies were subjected to biochemical analysis with multitest API® Biomerieux strips. Fungal colonies were identified to the level of genera by careful microscope analysis of fresh or lactophenol blue-stained preparations. Bacterial and fungal identifications were performed according to general procedures and taxonomic keys contained in established mannuals (Buchanan & Gibbons 1975, Samson & Van Reenen-Hoekstra 1988, Germain & Summerbell 1996, Prescott et al. 2004).

Results and Discussion

Microbial concentration of indoor air

Table 2 shows the mean values of the results obtained for bacteria and fungi in the air of each of the areas selected, as well as the total microbial concentration. As indicated, the values recorded in the study refer to two different periods of time, either in the absence of personnel (8 am) or in presence of users of the facilities inside the building (1 pm).

These data clearly reveal the presence of aerial

Point	Place	Bacteria (CFU/m³)		Fungi (CFU/m³)		Total (CFU/m³)	
		8:00 am	*1:00 pm*	*8:00 am*	*1:00 pm*	*8:00 am*	*1:00 pm*
1	Classroom 0.5	55	158	110	130	**165**	288
2	Classroom 0.6	110	168	105	105	215	273
3	Classroom 0.7	75	**338**	145	145	220	**483**
4	Aulario I Lecture room	**50**	118	205	120	253	238
5	Hall	163	198	225	220	388	418
6	Cafeteria	108	152	185	130	293	282
7	Library	78	126	110	135	188	261
8	Toilets	150	302	**275**	100	425	402
9	Reception	175	114	250	150	425	264
10	Office area	105	154	90	**70**	195	224
	Mean value	**107**	**183**	**170**	**131**	**277**	**313**

Tabla 2. Concentraciones microbianas en el aire del interior de la Facultad de Biología (CFU/m³). Los valores máximos y mínimos se indican en negrita. La desviación estándar en cada caso fue inferior al 10%.

Table 2. Microbial concentration in the indoor air of the Faculty of Biology (CFU/m3). Minimum and maximal values are indicated in boldface. Standard deviations were below 10%.

bacteria associated to the presence of personnel into the air of the partially closed premises. In fact, the mean value of viable bacteria almost doubled into the air from early morning till five hours later. When all the places under study were considered, the minimum for bacterial contamination (50 CFU/m³) was found in the absence of human activities, while the maximum value (338 CFU/m³) was detected in the presence of users of the facilities. Significantly, in most cases the major increases observed in bacterial concentration were associated to the number of occupants of the particular place (Table 2 and Fig. 1A), supporting the interpretation that the number of individuals into the facilities represents an important source of indoor contamination. Hence, an obvious practice to improve a more healthy quality of indoor air in the building would be to avoid crowding and to favour good ventilation.

Notably, the average fungal density found in the indoor air did not appear to follow the same trend than bacterial concentration. An overall view of the data indicated in Table 2 highlights that the bacterial microflora suspended into the air was predominant over the aerial indoor fungi (59% and 42% respectively) in the presence of human activities, while the reverse occurs in their absence. Although in some specific cases the values for fungal contamination were maintained or even increased during occupancy, its total mean concentration slightly decreased as occupation

progressed, suggesting that most fungal species present into the air were not human-borne. The temporal pattern of aerial fungal distribution was thus different, with minimal values in the presence of users (70 CFU/m³) and maximal concentration in the absence of human activities (275 CFU/m³) (Fig. 1B).

The above data are in agreement with previous observations by others and ourselves in the sense that, contrary to what happens in open air, bacterial concentration usually outnumbers fungal load in indoor environments (Ingold 1971, Atlas & Bartha 2002, Soto et al. 2009). It thus seems likely that the occupants of the building may be a source for the bacterial contamination, which can be dispersed through droplets during coughing, sneezing or skin peeling and then maintained in aerial suspension.

Indoor/outdoor ratio for bacteria and fungi

To calculate the indoor/outdoor ratio (I/O), reference samples were also taken from the external air in three areas close to the building and their microbiological content compared with the average counting values for bacteria and fungi inside the building. The level of bacterial and fungal contamination found in the outdoor air was rather similar to the detected in other urban areas of the city of Murcia (Soto et al., 2009). As Table 3 shows, the I/O ratio for bacteria clearly surpassed a value of 1 by almost 2-fold, again suggesting the

A

BACTERIA

B

FUNGI

Sample points
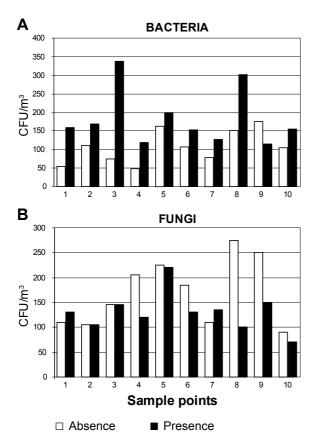
☐ Absence   ■ Presence

Figura 1. Valores medios de determinación de viables para bacterias (A) y hongos (B) en cada uno de los puntos de muestreo en ausencia (8 h) o presencia (13 h) de ocupación humana.

Figure 1. Mean values of bacterial (A) or fungal (B) counting for each sampling point in the absence (8 h) and presence (13 h) of users in the building.

	Bacteria	Fungi
Indoor	145 (CFU/m^3)	150 (CFU/m^3)
Outdoor	73 (CFU/m^3)	346 (CFU/m^3)
I/O ratio	1,99	0,43

Tabla 3. Cocientes de concentración interna/externa para bacterias y hongos.
Table 3. Ratios indoor/outdoor for bacteria and fungi.

BACTERIAL GENERA	Frequency (%)
Micrococcus	38
Staphylococcus	20
Streptococcus	14
Bacillus	11
Neisseria	7
Acinetobacter	5
Pseudomonas	2
Corynebacterium	2
Enterobacter	1
FUNGAL GENERA	**Frequency (%)**
Cladosporium	46
Alternaria	18
Penicillium	18
Aspergillus	9
Yeasts (*Candida, Rhodotorula, Cryptococcus*)	9

Tabla 4. Frecuencia relativa de bacterias y hongos aislados.
Table 4. Relative frequency of isolated bacteria and fungi.

existence of a human contribution to the indoor increase. However, the I/O ratio for fungi was lower than the unit, supporting that indoor fungal species are mostly derived from exogenous origin.

Bacterial diversity

Attempts were performed to identify the bacteria from the colonies more often isolated in the air samples. About 85% of the isolates were gram-positive bacteria and only 15% stained as gram-negative cells. These results are consistent with the functional resistance conferred by the differential cell wall structure and composition, and the major proportion of the skeletal peptidoglycan component in the former group. On the other hand, morphological studies showed that cocci were predominant over bacteria with bacillary shape (79% versus 21%). Over four hundred isolated colonies were selected at random and biochemically characterized to establish an overall taxonomic classification of the airborne bacteria

at the level of genus. The results obtained are shown in Table 4. The most abundant bacteria corresponded to species of *Micrococcus*, *Staphylococcus* and *Streptococcus*, to a lesser extent to *Bacillus*, *Neisseria*, *Acinetobacter*, *Pseudomonas* and *Corynebacterium*. *Enterobacter* was also occasionally isolated as a representative member of the enterobacteriaceae family.

The above results reveal that the bacteria most often isolated are gram-positive cocci belonging to saprophytic microflora generally associated to human skin and mucosa, thereby suggesting that the main bacterial contamination suspended into the indoor air derives from human presence.

Fungal identification

By following a similar planning to the described for bacterial diversity, we performed a macro and microscope analysis (both in fresh and lactophenol blue-treated preparations) of the colonies isolated on solid Sabouraud media supplemented with

chloramphenicol. Most detected fungi were filamentous species with aerial growth and fluffy, dusty or cotton-like appearance, although a small number of unicellular forms (yeasts) was also isolated. Determination of properties such as size, shape, hyphal disposition and spore arrangement led to the results shown in Table 4 on the most common fungi and their relative abundance.

Noteworthy, some species of these fungi, like *Cladosporium*, *Alternaria*, *Penicillium* and *Aspergillus*, are recognized opportunistic pathogens for humans and often associated with clinical manifestations (alergy, rhinitis, asthma, conjunctivitis). Also, these microorganisms are considered potential candidates involved in the establishment of sick building syndromes (Jones, 1999; Schwab & Straus 2004). As Table 4 indicates, yeasts were isolated in our study with a frequency close to 9%. This result is coincident with observations made by other authors, which detect yeasts in most indoor air samples and, in some instances, even at higher rates (Wanner et al., 1993). *Candida*, *Rhodotorula* and *Cryptococcus* are lipophilic yeast able to colonize human skin and they form part of the normal microflora of mouth, skin and nails.

Final remarks: microbiological indoor air quality

Currently, there is not a precise legislation in Spain on the microbiological quality of the air, although AENOR has proposed a Technical Committee on Normalization (CTN 171) to elaborate guidelines on the quality of the indoor air. In this context, the European Community Commission has proposed five different categories to evaluate the level of microbial contamination in the indoor air of non-industrial environments (ECC 1993). These categories are outlined in Table 5.

Taking into account the global results obtained in this work, the average bacterial and fungal aerial indoor concentration in the building analyzed is close to 145 CFU/m^3 and 151 CFU/m^3, respectively. Consequently, in agreement with the indications steaming from the ECC in 1993, the indoor air of the Faculty of Biology, as a whole, shows an intermediate level of microbial contamination (100-500 CFU/m^3). However, there were specific areas where the microbial load was of higher quality, as in the case of Aulario I Lecture Room, which contained low bacterial contamination early

in the morning, or the Office zone, which showed low fungal contamination throughout all the daily periods analyzed (Table 2).

On the other hand, according to the instructions of the Biological Aerosols Committee of the American Conference of Governmental Industrial Hygienists (ACGIH 1999), the normal indoor aerial microflora should be qualitatively similar to, and quantitatively lower than the one of the open atmospheric outdoor air. In the building under study, the above guidelines are completely fulfilled with respect to fungal contamination since the I/O ratio was below 1 and, in addition, the results shown here are qualitatively quite similar to those obtained in a previous work performed on the outdoor air of urban areas of the city of Murcia (Soto et al., 2009). In contrast, such a situation was not found for airborne bacteria, which showed an I/O ratio surpassing 1. Most bacteria that were present into the air are often part of the normal human microflora. They are related to the skin and the upper respiratory tract, and may thus not represent a direct danger for health. However, high viable counting indoor versus outdoor is indicative of crowding and poor ventilation conditions that should be improved (Morey 1993).

Convincing evidence, supported by molecular hints, indicates that only a small percentage of microorganisms surviving in natural environments can be cultured (and hence numbered) in conventional and standard microbiological media (Amann et al. 1995). Therefore, the results presented here may be an under estimate of the real indoor aerial microbial contamination. This remark, coupled to the fact that more than 10 m^3 are daily inhaled by a human adult (Cardona 2003) leads to the conclusion that the airborne microbial intake per day of the occupants of the analyzed building might likely exceed by at least 10-fold the average

Contamination category	Bacteria (CFU/m^3)	Fungi (CFU/m^3)
Very Low	< 50	< 25
Low	< 100	< 100
Intermediate	< 500	< 500
High	< 2000	< 2000
Very high	> 2000	> 2000

Tabla 5. Categorías propuestas para la contaminación del aire interior (ECC 1993).

Table 5. Categories for indoor air in non-industrial environments (ECC 1993).

number of microorganisms expressed above. The control of the microbial load of the surrounding air is thus an important factor to establish the quality and health conditions of the services rendered by any public institution.

Acknowledgements

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