View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Repositório Científico do Instituto Politécnico de Lisboa

Assessment of fungal contamination in a group of Lisbon's Gymnasiums with a swimming Pool

Carla Viegas[*,1], Célia Alves[2], Elisabete Carolino[1], Catarina Pinheiro[2], Laura Rosado[2], Carlos Silva Santos[3]

[1]*Escola Superior de Tecnologia da Saúde de Lisboa - Instituto Politécnico de Lisboa*
[2]*Laboratório de Micologia - Instituto Nacional de Saúde Dr. Ricardo Jorge*
[3]*Escola Nacional de Saúde Pública - Universidade Nova de Lisboa*

*Corresponding author:
Carla Viegas, Escola Superior de Tecnologia da Saúde de Lisboa - Instituto Politécnico de Lisboa, Av. D João II, Lote 4.69.01, 1990 - 096 Lisboa; Ph. ++(351) 218 980 400; Fax ++(351) 218 980 460; e-mail: carla.viegas@estesl.ipl.pt

The study's main purpose was the assessment of the environmental fungal contamination, the exploration of possible associations between related environmental variables and the study of the relationship between fungal contamination of air and surfaces.
A descriptive study was developed based upon air and surfaces monitoring for fungal contamination in ten indoor gymnasiums with a swimming pool located in Lisbon's urban area. Fifty 200 litres air samples and 120 surface swabs were collected. Surfaces samples were collected before and after cleaning and disinfection and temperature and relative humidity values were registered during the collection period. Twenty five different species of fungi were identified in the air samples, being the three most commonly isolated genera the following: *Cladosporium* (36.6%), *Penicillium* (19.0%) and *Aspergillus* (10.2%). Thirty-seven different species of fungi were identified in the surface samples. *Fusarium sp*. was the most frequent genera before (19.1%) and after (17.2%) cleaning and disinfection. There was a significant association between the numbers of visitors and the fungal contamination determined in the surface samples ($p<0.05$). There was no significant association ($p>0.05$) between the contamination encountered in the air samples and the one registered in the surface samples and between the fungal contamination and the temperature or relative humidity measured on location. The data obtained enabled the assessment of the establishment's fungal contamination and led the authors to conclude, consequently, that physical activity, which generally promotes health, can in fact be challenged by this factor.

Key words: Gymnasiums, Swimming pools, Fungal Contamination, Air, Surfaces

Introduction

Fungi are ubiquous in indoor and external environments and their quantity and variety changes with the seasons, level of precipitation, relative humidity, wind, temperature and availability and composition of nutrients. In indoor environments the air and surface fungal contamination is influenced not only by temperature, relative humidity and air flux/drafting but also by the weather conditions, hygiene standards and human activity [Goyer et al., 2001].
To assess mycological contamination it's important to consider the fungal spore's dispersion on air and surfaces. Type of sporulation and spore characteristics must be considered (size, density, colony structure and rugosity) as well as additional environmental characteristics, such as surface vibrations, smoothness and role as substrate [Roussel et al., 2008].
Culturing air samples is usually the only parameter used to assess indoor fungal contamination [Srikanth et al., 2008]. However, surface analysis complements the air characterization and is used in order to identify contamination sources. It may also be used in order to evaluate the efficacy of surface cleaning and disinfection procedures [Stetzenbach et al., 2004].
In Portugal, the law-decree Nr. 79/2006, of the 4th of April, approved the regulation of energetic systems of acclimatization of buildings and determines that the maximum concentration of fungal colony forming units by cubic meter ($CFU \cdot m^{-3}$) should not exceed 500. For surface's fungal contamination no legal references exist. According to Rao et al. [Rao et al., 1996], quantity and quality studies must be done simultaneously as different fungal species influence health in different ways.
According to Leoni et al. [Leoni et al., 1999] the microbiologic condition of a gym with at least one swimming pool should be frequently checked and new microbiologic indicators suggested. Lee et al. [Lee et al., 2004] refer fungi as biomarkers for environmental quality, especially for gyms with a pool [Brandi et al., 2007].
This study aimed not only to characterise fungal contamination of gyms with swimming pools but also to assess how this combination relates to other environmental variables and the way air and surface fungal contamination relate with each other.

Methods

A similar group of 10 indoor gyms with swimming pool (the busiest of the 30 existing in Lisbon and outskirts) were assessed once for fungal contamination of air and surfaces during the duration of this study (one year). Both structural conditions and cleaning procedures (products and techniques) are the same in all of the chosen establishments.
Using a Millipore Air Tester, 50 air samples of 200 litres each (140 $l \cdot min^{-1}$ flow) were collected in the area surrounding the swimming pool, training rooms, locker and shower rooms (male and female areas). An outdoor sample was also taken to be used as a fungal reference of the geographical area where the gym is located. Sampling took place at the height of 1 meter from the floor and after 21:00 hours to ensure the sampling was done at the worst possible scenario.
The air sampling equipment was calibrated (yearly) and wiped clean (before each use) with a gauze and a 70% ethanolic solution, as indicated by the Portuguese Standard NT-SCE-02 [2009]. The growth media used in the sampling was malt agar (MEA) with cloramphenicol. Environmental parameters (temperature and relative humidity) were determined with the Babuc A - LSI Systems equipment according to the International Standard ISO 7726:1998.
Surface sampling was done before and after cleaning and disinfection procedures, in 60 locations, totalizing 120 samples (six from each of 10 gymnasiums at the following sites: the floor surrounding the swimming pool; the floor surrounding the Jacuzzi; the stair's access to the swimming pool area; the floor of the training studios where most of the barefoot activities are performed and the changing/locker rooms from each gender).

© The Italian Association of Industrial Hygienists - AIDII [2011]

Swab technique was performed following the ISO 18593:2004, using a 10 by 10 cm steel stencil square cleaned with a 70% ethanol/water solution between samplings. All samples were kept refrigerated during transport and were processed on the same day. Swabs were streaked in triplets on MEA and Mycobiotic agar (MA) for dermatophyte growth. Plates were incubated at 27 ºC during 5 to 7 days for MEA and 15 to 20 days for MA and checked periodically to avoid over-growth.

Fungal identification by microscopic observation of morphologic characteristics using an identification Atlas [Hoog et al., 2000] was, whenever possible, performed to the species level, as health effects frequently vary within the same genus [Rao et al., 1996]. Yeast identification was achieved using bioMérieux ID 32C® [Ghannoum et al., 2000]. Quantitative results were expressed as the number of colony forming units per square meter (CFU·m^{-2}) for surfaces and per cubic meter (CFU·m^{-3}) for air.

Confidentiality and anonymity were ensured for all the data generated during this study.

Statistical analysis was done using the SPSS (version 17.0) and the statistical techniques used were: i) descriptive statistics (through frequency tables and graphs), ii) linear regression (to examine the influence of independent variable - referred to as X - in the dependent variable - referred to as Y - and explanatory power (in%) and Pearson correlation coefficient (to assess the shape and intensity of the relationship between two variables), iii) Wilcoxon test (to compare the before and after cleaning).

The influence of the number of users in the average fungal contamination of the air was not statistically significant ($p = 0.148 > 0.05$ - Figure 1) as it accounted for only 24.27% of total influences found.

Results

Air fungal contamination

Twenty five species of moulds were isolated and identified. Of these, 6 genera were isolated more frequently: (*Cladosporium* (36,6%), *Penicillium* (19,0%), *Aspergillus* (10.2%), *Mucor* (7%), *Phoma* e *Chrysonilia* (3.3%). The following species of *Aspergillus* were isolated: *A. flavus*, *A. niger*, *A. glaucus*, *A. fumigatus*, *A. parasiticus*, *A. restrictus* and *A. sydowii*. Other genera isolated were *Fusarium*, *Chaetomium*, *Acremonium*, *Arthrinium*, *Scytalidium*, *Bipolaris*, *Phialophora*, *Ulocladium*, *Paecilomyces* and *Ochroconis*. Concerning yeasts, *Rhodotorula sp.* (70%) *Trichosporon mucoides* (10%) and *Cryptococcus unigutulattus* (10%) were isolated (Table 1).

Table 1: Fungal species most frequently isolated in indoor air samples of the 10 gymnasiums monitored in this study

Moulds	Frequency (%)	Minimum - Maximum (CFU·m^{-3})
Cladosporium sp.	36.6	5 - 65
Penicillium sp.	19.0	5 - 25
Aspergillus sp.	10.2	5 - 30
Mucor sp.	7	5 - 40
Phoma sp.	3.3	5 - 30
Chrysonilia sp.	3.3	5 - 5
Others	20.3	-
Yeasts	**Frequency (%)**	**Minimum - Maximum (CFU·m^{-3})**
Rhodotorula sp.	70	5 - 15
Trichosporon mucoides	10	5
Cryptococcus unigutulattus	10	5
Others	10	-

Outside the buildings, the three most frequent genera isolated were also *Cladosporium* (50%), *Penicillium* (19.1%) and *Aspergillus* (6.9%). The only quantitative discrepancy found between indoor and outdoor air was in the women's shower/locker room of one of the gymnasiums and in the men's shower/locker room of a different gymnasium. In these cases, the indoor CFU·m^{-3} was higher than the outdoor one.

In at least one room of all the 10 gymnasiums there was a different fungal species from the ones isolated in the air surrounding the building. Some of the moulds found only in the interior were *Scytalidium sp.*, *Paecilomyces sp.*, *Phialophora sp.*, *Bipolaris sp.*, *Aspergillus sydowii*, *Ochroconis sp.*, *Cryptococcus unigutulattus* and *Rhodotorula sp.*

No statistically significant relation was found between other monitored parameters (temperature and relative humidity) and fungal contamination ($p > 0.05$).

The influence of the number of users in the average fungal contamination of the air was not statistically significant ($p = 0.148 > 0.05$ - Figure 1) as it accounted for only 24.27% of total influences found.

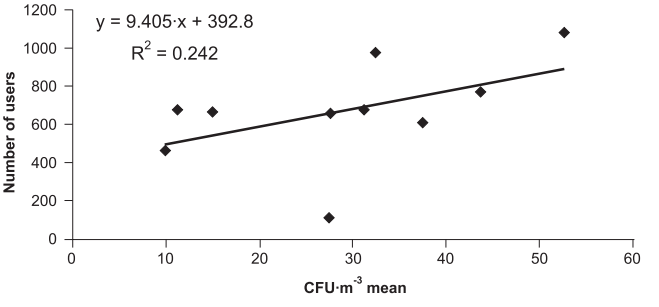

Figure 1: Influence determination of the total number of users of each gymnasium in the mean fungal counts.

Surfaces fungal contamination

Thirty seven different species of moulds were isolated and the most frequent genera before and after cleaning and disinfection were *Fusarium*, *Penicillium* and *Scytalidium*. Twenty nine species were isolated before cleaning and disinfection. In its genera, *Fusarium oxysporum* was the most frequently isolated species and *A. versicolor* was the most common species of *Aspergillus*, *T. mentagrophytes*, a *dermatophyte*, was also isolated. After cleaning and disinfection 25 different species were isolated, amongst which the most frequent within the genus *Fusarium* was also *Fusarium oxysporum*. As for *dermatophytes*, both *T. mentagrophytes*, and *T. rubrum* were isolated.

Twelve species of yeasts were isolated in total, of which *Cryptococcus* was the most frequent genus before the cleaning and disinfection and *Candida* was the most frequently isolated after this procedure.

Ten different species of yeasts were isolated before cleaning and disinfection: *Trichosporon mucoides*, *Rhodotorula sp.*, *Candida sp.*, *Candida parapsilosis*, *Candida guilliermondii*, *Cryptococcus humicola*, *Cryptococcus curvatus*, *Cryptococcus laurentii*, *Cryptococcus albidus* and *Cryptococcus uniguttulatus*. After the cleaning procedures, *Candida famata* and *Cryptococcus neoformans* were added to the list of fungi mentioned above (only *Cryptococcus curvatus* was not present at this moment) (Table 2). *Trichosporon mucoides* was the most frequently isolated both before and after disinfection.

© The Italian Association of Industrial Hygienists - AIDII [2011]

Table 2: Genera of moulds and yeasts most frequently isolated on the floor of ten gymnasiums

Moulds	Before cleaning and disinfection Frequency (%)	After cleaning and disinfection Frequency (%)
Fusarium sp.	19.1	17.2
Penicillium sp.	11.5	16.9
Scytalidium sp.	11.5	13.3
Phoma sp.	10.7	10.3
Cladosporium sp.	8.4	3.3
Aspergillus sp.	6.1	4.2
Trichophyton sp.	2	1.1
Others	30.7	33.7
Yeasts	**Before cleaning and disinfection Frequency (%)**	**After cleaning and disinfection Frequency (%)**
Cryptococcus sp.	40.6	7.8
Candida sp.	25.1	49.3
Trichosporon sp.	21.7	37.1
Rhodotorula sp.	12.6	5.8

In five out of the 60 monitored locations no fungi could be found. Comparing the CFU·m^{-2} found before and after the cleaning procedures allowed the authors to conclude that from the 55 places where fungal isolation was possible, in 26 of them (47.3%) there was a higher count after the cleaning procedures had taken place and only in 23 places (41.8%) was the situation reversed. In six locations (10.9%) there was no change in the number of CFU before and after cleaning and disinfection. It was also possible to verify a higher presence of yeasts than filamentous fungi both before and after cleaning.
The Wilcoxon test was applied to each of the six sampling locations in all 10 establishments studied to statistically compare the fungal contamination before and after cleaning and disinfection (Table 3).

Table 3: Wilcoxon test results: comparison of fungal contamination before and after cleaning procedures

Location	Molds		Yeasts	
	z	p	z	p
Mens changing/locker rooms	-1.442	0.149	-2.201	**0.028**
Womens changing/locker rooms	-0.184	0.854	-1.214	0.225
Access stairs to swimming pool area	-2.018	**0.044**	-0.105	0.916
Floor surrounding the swimming pool	-1.521	0.128	-0.365	0.715
Floor surrounding the Jacuzzi	-2.035	**0.042**	-0.980	0.327
Training studios	-1.300	0.194	0.000	1.000

There was a statistically significant decrease in the amount of filamentous fungi found after cleaning in the stairway leading to the pool area ($p = 0.0444 < \alpha = 0.05$) and in the Jacuzzi ($p = 0.042 < \alpha = 0.05$). Such a difference was also found in the number of yeasts found in the man's locker rooms ($p = 0.028 < \alpha = 0.05$). Considering the average values and the standard deviation results (which indicate high variability) from the CFU of filamentous fungi and yeasts before and after cleaning there was only a significant reduction in the number of yeasts found in the men's locker rooms (Table 3).

As verified in the fungal air contamination, there was also no statistically relevant association ($p > 0.05$) between the fungal contamination in the surfaces and the temperature or relative humidity values. The influence of the number of users using the establishments over the average yeasts and filamentous fungi CFU·m^{-2} before cleaning was also assessed. A statistically significant relationship ($p = 0.015 < 0.05$) was found between them and the total number of users contributes 65.31% to the explanation of variation of means of CFU·m^{-2} (Figure 2).

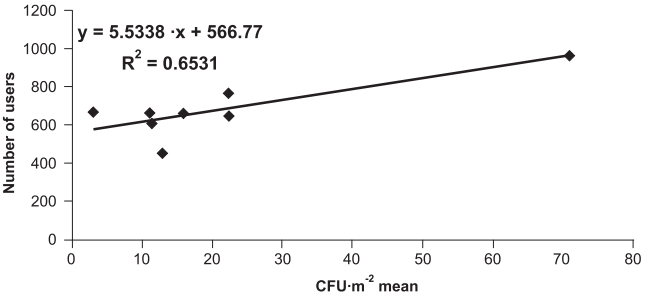

Figure 2: Simple linear regression and influence of the total number of users who attended each establishments and the CFU·m^{-2} mean before cleaning and disinfection.

Relationship between air and surfaces fungal contamination

There was no statistically relevant association ($p = 0.34 > 0.05$) between the surface contamination and the air contamination. In this case the air fungal contamination has only contributed with 1.91% to the surface fungal contamination (Figure 3).

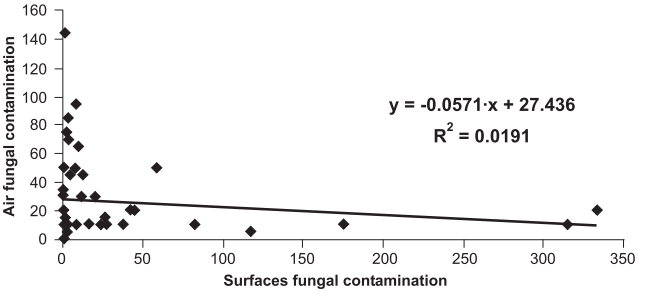

Figure 3: Simple linear regression and influence of air fungal contamination in surfaces fungal contamination.

A statistical analysis was also performed between air and surfaces fungal contamination. The Wilcoxon test was applied to these two variables and a statistically relevant association was found to a significance level of 10% ($p < 0.1$). Results are shown in Tables 4 and 5.

Table 4: Wilcoxon test application in fungal contamination of air and surfaces

	N	Orders means	Orders total
CFU·m^{-3} < CFU·m^{-2}	15	26.73	401.00
CFU·m^{-3} > CFU·m^{-2}	32	22.72	727.00
CFU·m^{-3} = CFU·m^{-2}	3		
Total	50		

Table 5: Wilcoxon test application in fungal contamination of air and surfaces

	CFU·m^{-3} - CFU·m^{-2}
Z	-1.726[a]
Asymp. Sig. (2-tailed)	**0.084**[b]

[a]Based on negative ranks; [b]Wilcoxon Signed Ranks Test

© The Italian Association of Industrial Hygienists - AIDII [2011]

Table 6 shows the median values and interquartile range. The median indicates that 50% of the lowest values are higher in the air fungal contamination (10 CFU·m^{-3} against 3 CFU·m^{-2}). Surfaces fungal contamination showed greater variability in 50% of core values (23.75 CFU·m^{-2} against 21.25 CFU·m^{-3}).

Table 6: Median and interquartile range of fungal contamination of air and surfaces

	Median	Interquartile range
CFU·m^{-2}	3	23.75
CFU·m^{-3}	10	21.25

The comparison between the surfaces and air fungal contamination is depicted in Figure 4. There were no significant differences between both values in spite of the presence of "outliers" in both data.

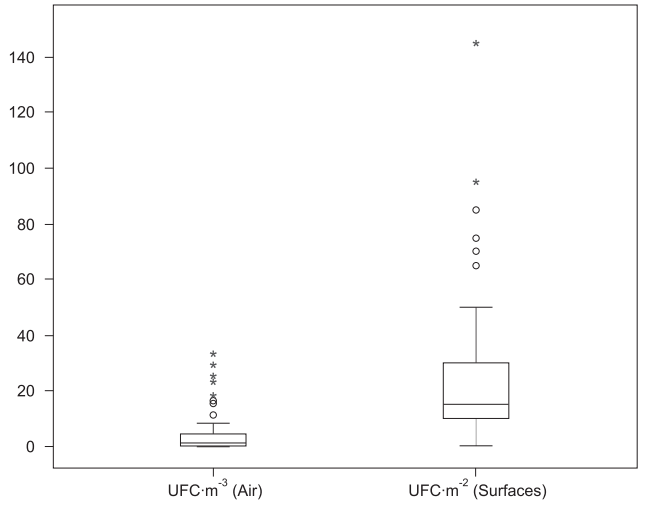

Figure 4: Comparison between surfaces fungal contamination and air fungal contamination (* severe outlier; o moderate outlier)

Discussion

Fungal species like *Aspergillus fumigatus*, *Aspergillus versicolor* and species from genera *Trichoderma*, *Penicillium*, *Phialophora*, *Fusarium* and *Ulocladium*, all of them isolated in the present study, are considered indicators of humidity problems and present a potential health hazard [Goyer et al., 2001] when present as air contaminants.

According to the 1996 American Industrial Hygiene Association (AIHA) guide for biologic contamination in air samples, the confirmed presence of *Stachybotrys chartarum*, *Aspergillus versicolor*, *Aspergillus flavus*, *Aspergillus fumigatus* or *Fusarium moniliforme* requires the enforcement of corrective measures. In the present study, the three mentioned *Aspergillus* species were found in the women and men's locker rooms and in the pool of more than one of the monitored gymnasiums. In terms CFU·m^{-3}, Miller et al. [Miller et al., 1998] have proposed the need for corrective measures whenever one or more of the following conditions are met in the same room: a) > 50 CFU·m^{-3} of a single species; b) > 150 CFU·m^{-3} in case of several species; c) > 300 CFU·m^{-3} when filamentous fungi are mainly present. The first condition was met with *Cladosporium sp*. in the male locker rooms in two of the studied gyms. Conditions b) and c) did not occur.

The World Health Organization (WHO) considers 150 CFU·m^{-3} a reason for concern, especially when potentially pathogenic species are found [Goyer et al., 2001] and this suggests the inadequacy of some of the species found in this study.

Other authors suggest different limits: Reynolds et al. [Reynolds et al., 1990] - 500 CFU·m^{-3}; Godish [Godish, 1991] - 1000 CFU·m^{-3}; Yang, Hung, Lewis and Zampiello [Yang et al., 1993] - 200 CFU·m^{-3}; Hurts et al. [Hurts et al., 1997] - 100 CFU·m^{-3}; Robertson [Robertson, 1997] - 300 CFU·m^{-3} and none of these were crossed in any of the monitored establishments. The results are also acceptable under the Portuguese legal requirement (500 CFU·m^{-3}).

According to Rao et al. [Rao et al., 1996] when there are governmental guidelines to assess the fungal contamination in air samples and, hence, its quality, these are normally not based in any health effects and are either absolute (numeric), relative (inside vs. outside) or a combination of both.

In the air, the levels of fungal contamination can go from 100 CFU·m^{-3} to 1000 CFU·m^{-3}. These can be considered low (1 to 499 CFU·m^{-3}), average (500 ÷ 999 CFU·m^{-3}) or high (> 1000 CFU·m^{-3}). According to these guidelines, all of the monitored locations have shown a low level of contamination. Nevertheless, when considering the quotient between the inside levels and the outside levels (and this value should be less than 1), one of the establishments presented a quotient higher than 1 and, therefore, one can predict the existence of interior sources of contamination.

Indoor air contamination, when significantly different from outside, may translate infiltration problems and potential health effects [Kemp et al., 2002]. The Portuguese legislation (NT-SCE-02, 2009) adopts a few of the criteria established by Miller et al. [Miller et al., 1998] and also suggests the lack of air quality when the fungal concentration inside is higher than outside. This was the case in two of the monitored locations. Species like *Aspergillus fumigatus*, *Aspergillus versicolor*, *Aspergillus flavus*, *Aspergillus niger* and genera *Fusarium* were found inside the monitored gymnasiums. According to the given legislation this means lack of air quality.

According to Nevalainen [Nevalainen, 2007] the exterior air is one of the main sources of fungal contamination which justifies the coincidental species found inside and outside. However, in one or more locations of all the 10 studied establishments some fungal species found inside were not identified outside, suggesting inside contamination [Kemp et al., 2002].

Regarding the results obtained when assessing the surfaces fungal contamination, it is important to highlight that some of the fungi isolated in this study are responsible for dermatomycosis, namely *T. mentagrophytes* and *T. rubrum* [Ali-Shtayeh et al., 2002], *Fusarium sp.*, *Scytalidium sp.*, *Aspergillus sp.*, *Cladosporium sp.*, *Phoma sp.* [Gianni et al., 2000], *Candida sp.*, *Trichosporon sp.* and *Cryptococcus sp.* [Araújo et al., 2003].

It was also possible to verify that in 26 from the 55 locations contaminated with fungi a higher number of CFU·m^{-2} was found after the cleaning and disinfection procedures and only in the men's locker rooms and only regarding yeasts was this situation reversed. This fact renders inefficient the cleaning process and can be due to inadequate procedures or use of inadequate cleaning products. Cross contamination can also occur due to the use of the same cleaning materials in other establishments. These incorrections can also account for the finding of different fungal species before and after the cleaning procedures.

Overall, the yeasts CFU·m^{-2} was higher than the filamentous fungi and this can be explained with the higher resistance opposed by these unicellular organisms to the cleaning procedures [Goyer et al., 2001].

© The Italian Association of Industrial Hygienists - AIDII [2011]

Due to their spore's nature, air dissemination is much more difficult for yeasts than for filamentous fungi [Goyer et al., 2001] and this can account for their minimal numbers in the air samples performed in the gyms. According to Kemp et al. [Kemp et al., 2002], concentration and type of spore is vital when identifying a species since these factors play an important role in air and surface dispersion.
In disagreement with international studies, the authors found no statistically relevant association between the air and surface fungal contamination and the temperature and humidity values ($p > 0.05$).
As published by Klánová and Hollerová [Klánová and Hollerová, 2003] but in disagreement with Lu et al. [Lu et al., 2009] and Brenier-Pinchart et al. [Brenier-Pinchart et al., 2009], the authors found no correlation between the quantitative data retrieved from the air and surface samples in all 10 establishments under study. The choice to assess both air and surfaces was proven correct in our case since without it no suitable environment quality assessment could have been done.
In the present study, the fungal distribution in air and surfaces was not similar, because it depends on fungal spores dispersion which varies with the fungal characteristics and environmental variables [Buttner and Stetzenbach, 1993; Kemp et al., 2003; Górny, 2004; Roussel et al., 2008].
There were significant differences (CI 90%, ($p < 0.1$)) between the fungal contamination present in the surfaces and air samples. As published by Brenier-Pinchart et al. [Brenier-Pinchart et al., 2009], half of the lowest values were higher in the air samples.
Nevertheless, the surface fungal contamination shower higher variability when compared with the air samples which reinforces the need for constant monitoring of surfaces in this specific professional setting.
The authors found no statistically relevant association between the total number of users in each of the gymnasiums studied and the average CFU determined in the air samples ($p > 0.05$). Extrinsic and intrinsic factors may be accountable for this lack of association [Roussel et al., 2008]. Nevertheless, there was a statistically significant association between the total number of users for each establishment and the average number of $CFU \cdot m^{-2}$ before the cleaning and disinfection procedures which can happen because the working staff and users of these gyms may carry several fungal species in their shoes [Scheff et al., 2000]. The fact that no relevant association could be found between the fungal contamination in air and surface samples ($p > 0.05$) confirms the need to assess them both in order to fully and suitably evaluate any given setting [Klánová and Hollerová, 2003].

Conclusions

The authors found no statistically relevant association between the air fungal contamination and the surface fungal contamination and no relevant association between these two variables and the temperature or relative humidity values. There was only a statistically relevant association between the total number of users and the average surface CFU before the cleaning and disinfection procedures.
The study made it possible to conclude: 1) the existence of fungal species in the air which demand corrective measures; 2) the presence, inside the 10 studied establishments, of fungal species different from the ones found outside; 3) the existence, in surfaces, of fungal species potentially responsible for dermatomycoses; 4) the cleaning and disinfection procedures fall below the expectations as far as elimination of the fungal species present is concerned. Also worth mentioning is the fact that two establishments presented a higher number of CFU than the outside sample used for comparison and two other establishments present more than 50 $CFU \cdot m^{-3}$ of the genera *Cladosporium*.
The complexity of a study on fungal contamination recommends the joint monitoring of both air and surfaces. Considering the results, it was possible to analyze the fungal contamination of the establishments and, as such, conclude that this contamination can undermine the health promoting effect intended with the practice of physical activity.

Bibliografia

Ali-Shtayeh, M., Khaleel, T., Jamous, R., 2002. *Ecology of dermatophytes and other keratinophilic fungi in swimming pools and polluted and unpolluted streams*. Mycopathologia, **156**, 193 - 205.

American Industrial Hygiene Association, 1996. *Field Guide for the Determination of Biological Contaminants in Environmental Samples*. AIHA.

Araújo, A., Sousa, M., Bastos, O., Oliveira, J., 2003. *Onicomicoses por fungos emergentes: análise clínica, diagnóstico laboratorial e revisão*. An. Bras. Dermatol., **78**, 455.

Brandi, G., Sisti, M., Paparini, A., Gianfranceschi, G., Schiavano, G., De Santi, M., Santoni, D., Magini, V., Romano-Spica, V., 2007. *Swimming Pools and Fungi: An Environmental epidemiology Survey in Italian Indoor Swimming Facilities*. Int. J. .Environ. Heal. R., **17**, 197 - 206.

Brenier-Pinchart, M., Coussa, R., Lebeau, B., Mallaret, M., Bulabois, C., Ducki, S., Cahn, J., Grillot, R., Pelloux, H., 2009. *Mobile air-decontamination unit and filamentous fungal load in the hematology ward: How efficient at the low-activity mode?* Am. J. Infect. Control, 1 - 3.

Buttner, M., Stetzenbach, L., 1993. *Monitoring Airbone fungal spores in an experimental indoor environment to evaluate sampling methods and the effects of human activity on air sampling*. Appl. Environ. Microb., 219 - 226.

Ghannoum, M., Hajeh, R., Scher, R., Konnikov, N., Gupta, K., Summerbell, R., Sullivan, S., Daniel, R., Krusinski, P., Fleckman, P., Rich, P., Odom, R., Aly, R., Pariser, D., Zaiac, M., Rebell, G., Lesher, J., Gerlach, B., Ponce-de-Leon, G., Ghannoum, A., Warner, J., Isham, N., Elewski, B., 2000. *A Large-scale North American Study of Fungal Isolates from Nails: The Frequency of Onychomycosis, Fungal Distribution and Antifungal Susceptibility Patterns*. J. Am. Acad. Dermatol., **43**, 641 - 648.

Gianni, C., Cerri, A., Crosti, C., 2000. *Non-dermatophytic onychomycosis. An underestimated entity? A study of 51 cases*. Mycoses, **43**, 29 - 33.

Godish, T., 1991. *Indoor Air Pollution Control*, Lewis Publishers.

Górny, R., 2004. *Filamentous microorganisms and their fragments in indoor air - A review*. Ann. Agric. Environ. Med., **11,** 185 - 197.

Goyer, N., Lavoie, J., Lazure, L., Marchand, G., 2001. *Bioaerosols in the Workplace: Evaluation, Control and Prevention Guide*. Institut de Recherche en Santé et en Sécurité du Travail du Québec.

Hurts, C., Walter, M., Stetzenbach, L., 1997. *Manual of Environmental Microbiology*. ASM Press.

Hoog, C., Guarro, J., Gene, G., Figueiras, M., 2000. *Atlas of Clinical Fungi. 2ª ed.* Centraalbureau voor Schimmelcultures.

Kemp, P., Neumeister-Kemp, H., Esposito, B., Lysek, G., Murray, F., 2003. *Changes in airborne fungi from the outdoors to indoor air; Large HVAC systems in nonproblem buildings in two different climates*. Am. Ind. Hyg. Assoc. J., **64**, 269 - 275.

Kemp, P., Neumeister- Kemp, H., Murray, F., Lysek, G., 2002. *Airborne fungi in non-problem buildings in a southern-hemisphere Mediterranean climate: preliminary study of natural and mechanical ventilation*. Indoor Built Environ., **11**, 44 - 53.

Klánová, K., Hollerová, J., 2003. *Hospital indoor environment: screening for micro-organisms and particulate matter*. Indoor Built Environ., **12**, 61 - 67.

Leoni, E., Legnani, P., Guberti, E., Masotti, A., 1999. *Risk of Infection Associated with Microbiological Quality of Public Swimming Pools in Bologna, Italy*. Public Health, **113**, 227 - 232.

© The Italian Association of Industrial Hygienists - AIDII [2011]

Lu, Z., Lu, W., Zhang, J., Sun, D., 2009. *Microorganisms and particles in AHU systems: Measurement and analysis*. Build. Environ., **44**, 694 - 698.

Miller, J., Laflamme, A., Sobol, Y., Lafontaine, P., Greenhalgh, R., 1998. *Fungi and fungal products in some Canadian houses*. Int. Biodeterior., **24**, 103 - 120.

Nevalainen, A., 2007. *Bio-aerosols as exposure agents in indoor environment in relation to asthma and allergy*. Section 3 Asthma and Allergy. Proceedings of the First ENVIE Conference on Indoor Air Quality and Health for EU Policy, Helsinki, Finland.

Rao, C., Burge, H., Chang, J., 1996. *Review of quantitative standards and guidelines for fungi in indoor air*. J. Air Waste Manage. Assoc., **46**, 899 - 908.

Reynolds, S., Streifel, A., Mcjilton, C., 1990. *Elevated airborne concentrations of fungi in residential and office environment*. Am. Ind. Hyg. Assoc. J., **51**, 601 - 604.

Robertson, L., 1997. *Monitoring viable fungal and bacterial bioaerosol concentrations to identify acceptable levels for common indoor environments*. Indoor Built Environ., **6**, 295 - 300.

Roussel, S., Reboux, G., Bellanger, A., Sornin, S., Grenouillet, F., Dalphin, J., Piarroux, R., Millon, L., 2008. *Characteristics of dwellings contaminated by moulds*. J. Environ. Monit., **10**, 724 - 729.

Scheff, P., Pulius, V., Curtis, L., Conroy, L., 2000. *Indoor air quality in a middle school, Part II: Development of emission factors for particulate matter and bioaerosols*. Appl. Occup. Environ. Hyg., **15**, 835 - 842.

Srikanth, P., Sudharsanam, S., Steinberg, R., 2008. *Bio-aerosols in indoor environment: Composition, health effects and analysis*. Indian J. Med. Microbi., **26**, 302 - 312.

Stetzenbach, L., Buttner, M., Cruz, P., 2004. *Detection and enumeration of airborne biocontaminants*. Curr. Opin. Biotech., **15**, 170 - 174.

Yang, C., Hung, L, Lewis, F., Zampiello, F., 1993. *Airborne fungal populations in non-residential buildings in the United States*. Proceedings of the 6th International Conference in Indoor Air Quality and Climate, **4**, 219 - 224.

 © The Italian Association of Industrial Hygienists - AIDII [2011]