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# Prevalence of Fungi in Indoor Air with Reference to Gymnasiums with Swimming Pools

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**Key Words**
Environmental variables · Fungal contamination · Gymnasiums · Humidity · Swimming · Temperature

**Abstract**
Fungal contamination of air in 10 gymnasiums with swimming pools was monitored. Fifty air samples of 200 L each were collected, using a Millipore air tester, from the area surrounding the pool, in training studios, in showers and changing rooms for both sexes, and also, outside premises, since these are the places regarded as reference. Simultaneously, environmental parameters – temperature and humidity – were also monitored. Some 25 different species of fungi were identified. The six most commonly isolated genera were the following: *Cladosporium* sp. (36.6%), *Penicillium* sp. (19.0%), *Aspergillus* sp. (10.2%), *Mucor* sp. (7%), *Phoma* sp. and *Chrysonilia* sp. (3.3%). For yeasts, three different genera were identified, namely, *Rhodotorula* sp. (70%), *Trichosporon mucoides* and *Cryptococcus uniguttulattus* (10%).

## Introduction

Indoor air quality is influenced by an unknown number of factors. Many of these are related to the structure and decoration of the building, its ventilation, internal temperature and humidity plus ingress of pollution from outside, and inevitably, contamination by microorganisms, especially fungi. Colonisation by microorganisms, their speciation and quantity, is dependent on the indoor microclimate. They require ideal conditions of temperature, humidity, oxygen, carbon sources, nitrogen and minerals to thrive. Their biological activity, seen as biodegradation and biodeterioration, depends on their enzyme activities, the environmental conditions, the competition phenomenon and the nature of the substrate on which they grow. In places where the fungal concentrations are high, they can cause the onset of symptoms and disease in people, particularly those who suffer from respiratory problems or have a weak immune system. The health effects are dependent on the species present, the concentration of their metabolic products and duration of exposure and individual susceptibility [1].


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Until now, epidemiological studies have failed to establish a causal relationship between the extent of fungal presence, exposure time and specific effects on health or frequency and the severity of symptoms reported. Studies tend to show only existence of a link between exposure to fungi and development of symptoms, especially respiratory symptoms [1]. However, fungal species have been generally identified as the cause of allergic diseases, headaches, eye irritation, obstruction of the airways, coughing and other symptoms.

It is necessary to point out that more than 80 fungal species are associated with allergic symptoms of the respiratory tract, and that almost all allergens are of fungal origin. The main causative genera are *Penicillium* sp., *Aspergillus* sp., *Cladosporium* sp. and *Alternaria* sp. [2]. In a study conducted in Italian swimming pools [3], the genera most frequently isolated were *Penicillium* sp. (33.7%), *Aspergillus* sp. (19.7%), *Cladosporium* sp. (19.7%) and *Alternaria* sp. (13.9%).

Air conditioning systems can reduce the number of fungal spores in indoor air by up to 50%. However, these systems can act, for some species, as reservoirs and carriers and can facilitate their spread, while for other species they can contribute effectively to the overall reduction or elimination [4]. Another aspect that may also help fungal development in indoor environments are building materials, since materials such as wood, plaster boards, cellulose, wallpaper, textiles, especially natural fibres are prone to fungal colonisation [5].

Regarding monitoring of indoor air quality, while there are no numerical criteria commonly accepted, interpretation of fungal levels should take into account contextual situations which state where sample collecting is done, as if the worst scenarios are selected to be monitored. There is a criterion proposed by the scientific community that relies upon the comparison between species and their concentrations found in the interior environment when compared to the exterior, or another location which serves as a reference, and is a valuable indication to assess whether there is contamination of the indoor environment. If indoor concentrations are significantly higher than outdoors or if the species found are different, existence of bioaerosols and/or local fungi proliferation, should be expected. In addition to outdoor concentrations, those obtained in control places or during a pause in a process or professional activity may also be used as reference [1].

Portuguese law approving regulation of energy systems for air conditioning in buildings provides, for indoor environments, maximum reference concentration of $500\,CFU{\cdot}m^{-3}$ in air, which is a much higher value than the threshold stipulated by the World Health Organization (WHO), $150\,CFU{\cdot}m^{-3}$, not mentioning the absence of criteria regarding fungal species identified.

Microbiological air quality from gyms with swimming pools must be constantly checked, which suggests that new microbiological indicators are needed to define hygienic conditions [6]. Thus, fungi may represent environmental quality biomarkers, especially in gyms with swimming pools [1].

In Portugal, in recent years, the prevalence of diseases such as asthma and rhinoconjunctivitis has increased from 15% to 25% and 10% to 15%, respectively, in the general population [7]. Various causes have been considered, including indoor air pollution caused by fungal contamination. Therefore, it is important to contribute to the increase of knowledge by referring to fungal contamination of indoor spaces such as gyms with swimming pools, in order to identify the most effective preventive measures to avoid excessive contamination.

This investigation was designed to describe the environmental fungal contamination phenomena in the air of gyms with swimming pool and to explore possible associations with independent environmental variables.

## Materials and Methods

Fungal contamination in air sampled from 10 gyms with swimming pools was monitored for this descriptive cross-sectional study. The establishments selected were the most popular of 30 existing in Lisbon.

Considering air sampling as the best way to characterise fungal contamination in a given area [8], a total of 50 air samples of 200 L each were collected, at $140\,L{\cdot}min^{-1}$, over 1 year, using the Millipore air tester equipment, from areas surrounding the pool, in training studios, in showers and changing rooms for both sexes, and also outside the premises, the results from which were regarded as a reference. Samples were taken at 1 m above ground or floor level and always in the evening, after 9 p.m., in order to monitor the worst scenario of fungal contamination. Simultaneously, two environmental parameters – temperature and humidity – were monitored using the Babouc equipment (LSI Systems), according to the International Standard ISO 7726.

After laboratory processing and incubation of the collected samples, quantitative ($CFU{\cdot}m^{-3}$) and qualitative results were obtained through identification of isolated fungal species. Whenever possible, filamentous fungi and yeasts were identified to the species level, since adverse

health effects vary with different fungal species [9]. Identification was achieved through morphological characteristics listed in illustrated literature [10–13].

All sites monitored had air conditioning and a ventilation system responsible for recycling/renewal and heating/cooling of air, but did not have any device for dehumidifying/humidifying the air. Cleaning and disinfection operations in the establishments were held after the facilities were closed.

With the data obtained, tables with the frequency distribution of isolated fungal species were made and dependence of fungal concentration on the two monitored environmental parameters – temperature and humidity – was analysed.

**Table 1.** Most frequently found filamentous fungi and yeasts isolated from indoor air of the 10 establishments monitored

| | Frequency (%) | Minimum–maximum ($CFU \cdot m^{-3}$) |
|---|---|---|
| Filamentous fungi | | |
| *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 | | |
| *Rhodotorula* sp. | 70 | 5–15 |
| *T. mucoides* | 10 | 5 |
| *C. uniguttulattus* | 10 | 5 |
| Others | 10 | – |

## Results

From the air samples, 25 different species of filamentous fungi were identified. Among these, six genera were isolated more often, particularly *Cladosporium* sp. (36.6%), *Penicillium* sp. (19.0%), *Aspergillus* sp. (10.2%), *Mucor* sp. (7%), *Phoma* sp. and *Chrysonilia* sp. (3.3%). Among *Aspergillus* sp. genus were identified the species *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus glaucus*, *Aspergillus fumigatus*, *Aspergillus parasiticus*, *Aspergillus restrictus* and *Aspergillus sydowii*. In addition to these, other genera were also identified: *Fusarium* sp., *Chaetomium* sp., *Acremonium* sp., *Arthrium* sp., *Scytalidium* sp., *Bipolaris* sp., *Phialophora* sp., *Ulocladium* sp., *Paecilomyces* sp. and *Ochroconis* sp. With regard to yeasts were identified three different genera, including *Rhodotorula* sp. with an isolation frequency of 70% and *Trichosporon mucoides* and *Cryptococcus uniguttulattus*, these two presenting with a frequency of isolation of 10% (Table 1).

Regarding the most frequently isolated fungal species in different areas monitored in gyms with swimming pool – male and female showers/changing rooms, pool surroundings and studio – the three most common were: *Cladosporium* sp. (33.3–56.25%), *Penicillium* sp. (14.3–29.6%) and *Aspergillus* sp. (6.86–15.8%). Besides these, *Mucor* sp. was also isolated with some expression (8.8%) in male showers/changing rooms. Yeasts have otherwise shown little expression in the air samples collected. Outside, the three genera with greater isolation frequency were also *Cladosporium* sp. (50%), *Penicillium* sp. (19.1%) and *Aspergillus* sp. (6.9%) (Table 2).

Regarding comparison of concentrations found, in this study, for indoor and exterior environments, only in male showers/changing rooms of the gym with pool no. 2 and in female showers/changing rooms of the gym with pool no. 6, monitoring carried out indoor produced more $CFU \cdot m^{-3}$ than the sampling carried out outdoors. In all the other monitored spaces, the indoor areas showed less contamination than the exterior areas, as presented in Figure 1. However, in all the 10 establishments' monitored, fungal species different from the ones isolated outdoors were present in one or more indoor areas. Some fungi and yeasts that were only isolated indoors were, *Scytalidium* sp., *Paecilomyces* sp., *Phialophora* sp., *Bipolaris* sp., *A. sydowii*, *Ochroconis* sp. and *C. uniguttulattus* and *Rhodotorula* sp.

Results related to the influence of the environmental variables monitored revealed a very weak positive correlation with temperature (not significant; $p > 0.05$), which varied in a positive direction (increase) of $0.1\,CFU \cdot m^{-3}$ and contributed only some 0.14% $CFU \cdot m^{-3}$ of the total variation. For relative humidity, it was found that for each additional unit in humidity, there is a change in the negative sense (decrease) of $0.203\,CFU \cdot m^{-3}$ (not significant; $p > 0.05$), contributing to 19.8% of the total $CFU \cdot m^{-3}$. These results were already expected, because in our study, temperature and humidity correlation with $CFU \cdot m^{-3}$ had been shown to be very weak, as shown in Figure 1 concerning male showers and changing rooms.

It was also possible to verify that when conditions optimal for fungal spreading were registered – temperature between 22°C and 27°C and humidity between 75% and 80% – in the male showers/changing rooms of gym with pool no. 3, the total concentration ($45\,CFU \cdot m^{-3}$) was lower than that outdoors. On the contrary, in establishment number 10, also in male showers and changing

**Table 2.** Predominant species of filamentous fungi and yeasts isolated in the air of the 10 establishments monitored

| Locale | Filamentous fungi | | | Yeasts | | |
|---|---|---|---|---|---|---|
| | Total CFU·m$^{-3}$ | Species | Frequency (%) | Total CFU·m$^{-3}$ | Species | Frequency (%) |
| Female showers and changing rooms | 315 | *Cladosporium* sp. | 33.3 | 40 | *Rhodotorula* sp. | 87.5 |
| | | *Penicillium* sp. | 20.6 | | | |
| | | *Aspergillus* sp. | 15.8 | | | |
| Male showers and changing rooms | 455 | *Cladosporium* sp. | 34.1 | 20 | *Rhodotorula* sp. | 50 |
| | | *Penicillium* sp. | 14.3 | | | |
| | | *Mucor* sp. | 8.8 | | | |
| Pool surrounding area | 135 | *Cladosporium* sp. | 40.7 | 5 | *Rhodotorula* sp. | 100 |
| | | *Penicillium* sp. | 29.6 | | | |
| | | *Aspergillus* sp. | 7.4 | | | |
| Training studios | 160 | *Cladosporium* sp. | 56.3 | 0 | – | – |
| | | *Penicillium* sp. | 21.9 | | | |
| | | *Aspergillus* sp. | 9.3 | | | |
| Reference local (outside) | 1020 | *Cladosporium* sp. | 50 | 10 | *Trichosporon* sp. | 100 |
| | | *Penicillium* sp. | 19.1 | | | |
| | | *Aspergillus* sp. | 6.9 | | | |

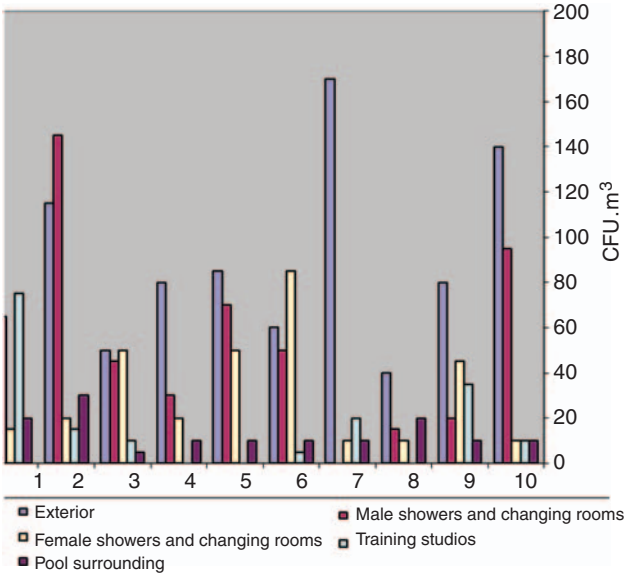


**Fig. 1.** Filamentous fungi isolated from indoor air and from the exterior of the 10 establishments monitored.

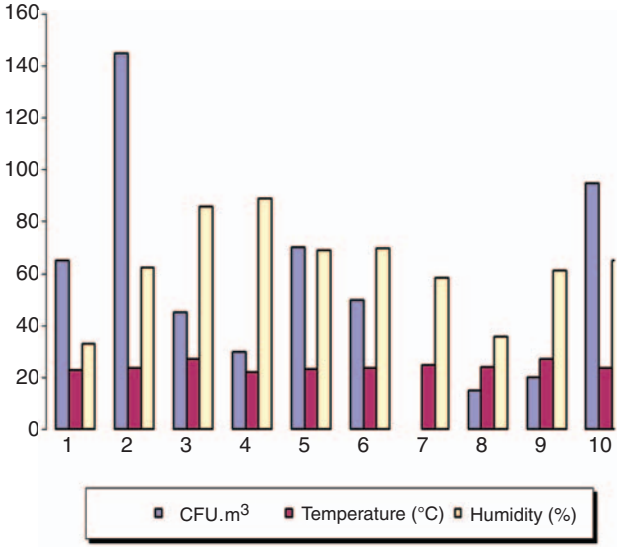


**Fig. 2.** Filamentous fungi isolated from the indoor air of the 10 establishments monitored related to environmental variables.

rooms, although temperature was within the preferred range for fungal development (23.8°C), the humidity suggested a negative impact on fungal spreading (estimated at 65.1%) and 95 CFU·m$^{-3}$ were isolated (Figure 2).

## Discussion

Considering the increasing demand for spaces where physical activity can be practised [3,14], the fact that fungi may represent effective biomarkers of environmental quality and that air samples are the best way to characterise fungal contamination of a given area [8], it is possible to illustrate the relevance of this study in gyms with swimming pools.

With regard to the qualitative assessment of fungal contamination, it is suggested that among other species, *A. fumigatus*, *Aspergillus versicolor* and *Penicillium*, *Trichoderma*, *Phialophora*, *Fusarium* and *Ulocladium* species, all isolated in this study, should be regarded as indicators of humidity problems and/or a potential risk to health [1]. Moreover, according to the American Industrial Hygiene Association (AIHA), in 1996, concerning the

determination of biological contamination in environmental samples, the confirmed presence of the species *Stachybotrys chartarum*, *A. versicolor*, *A. flavus*, *A. fumigatus* and *Fusarium moniliforme* requires implementation of corrective measures [15]. Confirmed presence of these species occurs when several colonies in different samples, several colonies in a single sample or even a single colony in a single sample, showing the growth of these species in construction materials are verified. In this study, the three species of *Aspergillus* mentioned above were identified in male and female showers/locker rooms and surrounding the pool, of more than one of the establishments monitored. Also noteworthy is the fact that *A. fumigatus*, one of the species isolated in the study, is one of the saprophytic fungi most widespread in air and is capable of causing severe or sometimes fatal aspergillosis [16].

The three genera isolated most often in this study, *Cladosporium* sp., *Penicillium* sp. and *Aspergillus* sp., were those found most frequently in other studies [17–19] including a study made in Lisbon [20].

For *Aspergillus* sp. and *Penicillium* sp., there are different potential risks associated with their inhalation, due to the release of toxins. In case of *Alternaria* sp. and *Cladosporium* sp., their simple presence can be an indicator of potentially pathogenic effects [21]. *Cladosporium* sp., the predominant genus in this study, with an indoor frequency of isolation of 36.6%, is probably the fungus that occurs most frequently around the world, especially in temperate climates [22]. The same genus is commonly connected to problems of indoor condensation [23].

From the quantitative assessment ($CFU \cdot m^{-3}$), we propose implementation of corrective measures whenever, in a given space, one or more of the following conditions are verified: (1) $>50\,CFU \cdot m^{-3}$ of a single fungal species; (2) $>150\,CFU \cdot m^{-3}$ if several fungal species are isolated; (3) $>300\,CFU \cdot m^{-3}$ if there are mainly filamentous fungi [24]. The first condition – (1) – was found for genus *Cladosporium* sp. in male showers/locker rooms of two of the monitored establishments. Neither condition (2), nor condition (3) occurred. If fungal concentration indoor is considerably lower than that outdoors, there should be no concerns about fungal contamination [21].

Quantitative guidelines outlined by government agencies, relating to fungi present in air are, rather than being based on health effects, usually absolute (numbers), relative (the ratio between indoor and outdoor levels) or a combination of both. Levels of fungal contamination in air can go from 100 to $1000\,CFU \cdot m^{-3}$. These can be considered low ($1–499\,CFU \cdot m^{-3}$), medium ($500–999\,CFU \cdot m^{-3}$) or high ($>1000\,CFU \cdot m^{-3}$). Given this criterion, all areas monitored in this study showed low levels of fungal contamination. Considering the ratio between levels of indoor and outdoor fungal contamination, there was one establishment for which a value higher than 1 was obtained, indicating therefore the existence of internal sources of contamination [9].

The WHO recognises that a level of $150\,CFU \cdot m^{-3}$ is a cause for concern, especially if potentially pathogenic species are found, and suggests that the presence of certain species is unacceptable, as mentioned earlier, in an indoor environment [1]. Other limits, higher than those found in the study, have been suggested by various authors, including: Holmberg [25], who supports the idea that concentrations below $2200\,CFU \cdot m^{-3}$ corresponds to indoor environments without fungal contamination; Morey et al. [26], who suggest that for concentrations of $1000\,CFU \cdot m^{-3}$ or more, it is necessary to investigate the possible sources of contamination; Ohgke et al. [27], who suggest that concentrations exceeding $100\,CFU \cdot m^{-3}$ denote sources of internal contamination and require detailed investigation; Burge [28], who recommends the need for further investigation into sources of internal contamination whenever interior levels are double the exterior levels and are higher than $1000\,CFU \cdot m^{-3}$; Reynolds et al. [29], who argue that concentrations exceeding $500\,CFU \cdot m^{-3}$ should be considered abnormal; Godish [30], who stipulates that values over $1000\,CFU \cdot m^{-3}$ signify a high fungal contamination; Yang et al. [31], who indicated $200\,CFU \cdot m^{-3}$ as a threshold value for indoor environments and implementation of corrective measures whenever this value is exceeded; Hurst et al. [32], who state that $100\,CFU \cdot m^{-3}$ is the limiting value for the occurrence of allergic reactions in occupied spaces; and Jo and Seo [19], who mentioned that if a concentration of $800\,CFU \cdot m^{-3}$ (value guide in Korea) is exceeded, sources of contamination should be investigated in order to proceed to their mitigation through implementation of corrective measures.

Taking into account what is mentioned in the Portuguese law, a level of $500\,CFU \cdot m^{-3}$ is the maximum reference concentration acceptable in interior air. This was not exceeded in any of the establishments monitored. Male showers/locker rooms in establishment no. 10, where $95\,CFU \cdot m^{-3}$ were isolated, revealed the highest fungal contamination.

As already mentioned, it is suggested that fungal levels found indoors should be compared quantitatively and qualitatively with those found outdoors, because the former is dependent on the latter [1]. Nevertheless, when

it comes to fungal levels, it should be taken into account that internal and external environments are quite different which, by itself, justifies diversity of species between different spaces. However, the fact that there is no stipulated limit with regard to fungal contamination makes it essential to compare fungal levels indoors and outdoors. Thus, indoor air quality that is significantly different from that outside could mean that there are infiltration problems and the potential exists for health effects. In this study, with regard to quantitative comparison, only in two locations did the monitoring that was carried out indoors show higher $CFU \cdot m^{-3}$ than that measured outside (Figure 1). At all other locations, monitoring carried out indoors showed fewer $CFU \cdot m^{-3}$ than in the samples from outdoors, suggesting that air conditioning filtering systems, which contribute to fungal species collection, retention and/or elimination, were working effectively [33].

It is worth mentioning that as outdoor air is a major source of the fungi found indoors, it is no coincidence that the prevailing genera, in this case *Cladosporium* sp., *Penicillium* sp. and *Aspergillus* sp., are the same in both these environments [34]. Nonetheless, all the 10 establishments monitored had one or more spaces with fungal species that differed from the ones isolated outside, suggesting fungal contamination from within.

Unlike the study held in Mexico City [21], where the most frequent fungal species found outdoor was *A. niger*, in this study *A. flavus* was the most frequent member of the *Aspergillus* genus which was isolated in both indoor and outdoor air, from the monitored establishments. This difference may be the result of different climatic conditions. It is also noteworthy that in this study, some species of same genus – *A. candidus* and *A. versicolor* – were only isolated outdoors, whereas others – *A. sydowii*, *A. restrictus*, *A. parasiticus* and *A. clavatus* – were only found indoors. Additionally, in some of the areas monitored, there were species of *Aspergillus* with higher concentration indoors than outdoors.

Studies on fungal spread in indoor environments give evidence of the presence of high levels of humidity, which agrees with the presence of fungi and may lead to biased conclusions. Most of the fungal species need moisture levels above 75% to grow, which is the reason for its development in kitchens and toilets [35]. Contrary to this, this study found a negative variation of fungal concentration with the level of moisture, suggesting that further investigation is necessary to ascertain the influence of this variable.

With regard to temperature, another environmental variable influencing fungal spreading, a study was conducted that showed peaks of fungal spores in outside air at temperatures between 22°C and 27°C and humidity between 75% and 80%, while fewer fungal spores were found with temperatures between 43°C and 48°C and humidity between 25% and 40% [36]. Nevertheless, we observed a weak positive correlation between fungal concentration and temperature; a result already foreseen, since in the only area where environmental conditions, in relation to temperature, were ideal for fungal spreading, fungal concentration was not the highest measured.

Results related to environmental variables addressed in this study are not consistent with the results of the other studies already mentioned. This may be justified by considering the effect of other environmental variables that also influence fungal spreading, namely, cleaning operations in the monitored areas, components of heating, ventilation and air conditioning systems (essential in gyms with swimming pools although these work as fungal reservoirs) and also users and physical activity professionals, who may carry, on their own body (commensal flora) or clothing, a great diversity of fungal species [37]. Besides this, activities as simple as walking in a monitored area may also affect fungal concentrations [38].

## Conclusions

With the data collected, it was possible to characterise fungal distribution in the air of different areas of the gyms with swimming pool and to evaluate the association of environmental variables with this distribution. The fungal genera most often isolated both indoors and outdoors were *Cladosporium* sp., *Penicillium* sp. and *Aspergillus* sp.

Quantitative measurements showed that our results, which were similar to those found by many other researchers, did not exceed the level suggested in the national law.

Also considering the ratio between the levels of fungal contamination indoors and outdoors and regarding the total amount of $CFU \cdot m^{-3}$, only one gymnasium with a pool presented a ratio greater than 1, indicating the existence of internal sources of contamination. Besides that, regarding quantitative comparison between concentrations indoors obtained in the different locations and outdoors, only in two places, belonging to different establishments, did the monitoring carried out indoors show more $CFU \cdot m^{-3}$ than outdoors.

However, all 10 establishments showed, in one or more of the spaces monitored, fungal species indoors that were

not isolated from the monitoring carried out outdoors, suggesting fungal contamination coming from within. Also relevant is the fact that some of the species present indoors – *A. fumigatus*, *A. versicolor* and species of *Trichoderma* and *Penicillium*, *Phialophora*, *Fusarium* and *Ulocladium* – are regarded as indicators of humidity problems and pose a potential health risk which could involve invoking corrective measures.

Unlike other studies, environmental variables monitored (temperature and humidity) did not show the expected association with fungal concentration, which may possibly have resulted from other confounding variables not investigated in this study, as for example, physical activities practised, number of users, effectiveness of ventilation and humidifying systems and the level of sanitation, among others.

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