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Characterizing the fungal and bacterial microflora and concentrations in fitness centres

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Abstract

Fitness centres are special places where conditions for microbiological proliferation should be considered. Moisture due to human perspiration and water condensation as a result of human physical activities are prevalent in this type of buildings. Exposure to microbial contaminants is clinically associated with respiratory disorders and people who work out in polluted environments would be susceptible to contaminants. This work studied the indoor air contamination in three gymnasiums in Lisbon. The sampling was performed at two periods: at the opening (morning) and closing (night) of the three gymnasiums. The airborne bacterial and fungal populations were sampled by impaction directly onto Tryptic Soy Agar (for bacteria) and Malt Extract Agar (for fungi) plates, using a Merck MAS-100 air sampler. Higher bacterial concentrations were found at night as compared to the morning but the same behaviour was not found for fungal concentrations. *Gram-negative catalase positive cocci* were the dominant bacteria in indoor air samples of the studied gymnasiums. In this study, 21 genera/species of fungal colonies were identified. *Chrysosporium* sp., *Chrysonilia* sp., *Neoscytalidium hialinum, Sepedonium* sp. and *Penicillium* sp. were the most prevalent species identified in the morning, while *Cladosporium* sp., *Penicillium* sp., *Chrysosporium* sp., *Acremonium* sp. and *Chrysonilia* sp. were more prevalent at night. A well-designed sanitation and maintenance program for gymnasiums is needed to ensure healthier space for indoor physical activity.

Keywords

Indoor air, Bacteria, Fungi, Fitness centres

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Introduction

Within indoor air, there is a complex mixture of viable and non-viable particles. The non-viable include inorganic particles, such as metals and other chemical compounds, and organic non-reactive material. The viable components are those that are capable of growing under favourable conditions, such as bacteria, fungi and all other microorganisms. Bioaerosols are normally defined as 'particles with biological origin suspended in the air', which can cause health effects, especially in the upper airways.[1–3]

The indoor microbial pollution involves hundreds of species of bacteria and fungi growing inside buildings when specific conditions are favourable. The main factors that influence microbial growth in a building are moisture, temperature and nutrient availability. The ventilation rate for air renewal is also a crucial

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factor for the control of microbial growth. In fitness centres, moisture due to perspiration and water condensation, marked human presence, elevated physical activity that promotes the resuspension of dust from the ground and contact between the occupants and surfaces (pavement, fitness equipment) are conditions that promote the microbial growth. Fungi are ubiquitous microorganisms that proliferate in more diverse environments due to their lower water activity (a_w) than bacteria. Bacteria require an a_w above 0.80, while fungi present minimum a_w of approximately 0.70.[4] Moreover, fungi are less selective in what concerns the substrate and consequently are able to grow on a diverse range of surfaces (wood, wall paper, etc.). Combined with these conditions for growth, joins the existence of fungal spore in indoor air. These spores are easily released into the air through aerial hyphae, while in the case of bacteria this process is not easy to promote, due to its gelatinous colonies.

Exposure to microbial contaminants is clinically associated with respiratory symptoms, allergies, asthma and immune reactions[5] depending upon the nature of the microbiological agent and the host's immune status. Some species of gram-negative bacteria are of most concern when present in indoor air because they are producers of endotoxins that can cause respiratory symptoms, including non-allergic asthma.[5] Gram-positive bacteria represent the largest group present in the atmosphere due to their greater resistance and survival abilities.[6,7] Fungi species among *Aspergillus, Penicillium* and *Fusarium* genera are producers of mycotoxins which can enter the human body by inhalation, dermal and oral contact, thereby causing different reactions in the host organism.[8]

Athletes and the common individual that practice sport present a higher risk of contact with bioaerosols and pollution due to the fact that

1. the minute ventilation could proportionally enhance the quantity of inhaled pollutants;
2. most of the air is inhaled through the mouth, bypassing the normal nasal mechanisms of filtration of larger particles and
3. the increased airflow velocity would carry pollutants deeper into the respiratory tract.[9]

However, despite the importance of healthy air in sport facilities, indoor air quality (IAQ) studies have been focused principally on schools,[10–15] elderly care centres,[16–19] homes[20] and offices.[21,22] Comparatively, IAQ evaluations carried out in fitness centres (not school gymnasiums) are very scarce and few have been reported.[23–26]

The aim of this work was to assess indoor air contamination in three gymnasiums, by fungal identification and bacterial characterization, in order to estimate the potential biological hazards during sporting activity in fitness centres.

Methodology

Sampling sites

In this study, three gymnasiums in the city of Lisbon were selected, and termed as follows: gymnasium 1 (G1), gymnasium 2 (G2) and gymnasium 3 (G3). Inside the fitness centres, sampling sites were chosen: the studios and the bodybuilding rooms. In G2 and G3, only the studio with the most practicing fitness classes was monitored, whereas in G1, two studios were evaluated. As described in Table 1, all fitness centres have identical location besides having a different surrounding. All fitness centres have mechanical ventilation; however, G2 preferentially uses natural ventilation rather than mechanical ventilation as it was observed that it was often switched off. The sampling campaigns were performed between October and December of 2012.

Air sampling

Samples were collected in two periods of the day - in the morning and at night (at the opening and closing of the gymnasium) - in order to recognize the differences before and after occupancy. Air samples were collected at the centre of the studied room, on ground level.

Air sampling was conducted using a microbial air sampler (MAS-100, Merck Millipore, Germany) that collected, by impaction, 0.25 m^3 of air in each plate, with a flow rate of 6 m^3/h. Two different culture media were used in order to provide to the microorganisms the most suitable nutrients for their growth: Malt Extract Agar (supplemented with 0.1 kg/m^3 chloramphenicol), used for fungi, and Tryptic-Soy Agar, used for bacteria. Tryptic-Soy Agar is a general agar medium used for culturing many kinds of non-fastidious and moderately fastidious microorganisms.[27] The sampling was also performed outdoors to compare the results between the indoor and the outdoor environments. The samples were sealed with parafilm and transported to the laboratory in a cooler bag. Air sample culture plates were incubated at 30 °C between 5 and 7 days (Memmert oven, Germany). A total of 48 Petri dishes with bacterial colonies and 48 Petri dishes with fungal colonies were analyzed. The colony counts were corrected using the positive hole correction table MAS-100, provided by the supplier.[28] The microbiological concentrations were expressed in colonies forming units per cubic metre ($CFU.m^{-3}$).

Table 1. Main characteristics of the studied gymnasiums.

Gym	Year of opening	Location	Site[a]	Area (m^2)	Volume (m^3)	Capacity (person)[b]	Sampling Days	Code	Cleaning Operations	Floor type	Wall type	Ventilation system
G1	2010	Urban (street with intense road traffic)	S1	149	447	35	1 day	G1S1	In the middle of morning and afternoon At the closing time	Wood	Brickwork Glass	Mechanical
			S2	263	788	35	1 day	G1S2				
			Bb	650	1948	60	2 days	$G1Bb_1$ $G1Bb_2$	At the closing time			
G2	2000	Urban (inside a city park)	S	240	1156	40	2 days	$G2S_1$ $G2S_2$	In the middle of the day At the closing time	Wood	Brickwork	Mixed (natural and mechanical)
			Bb	180	540	40	2 days	$G2Bb_1$ $G2Bb_2$	At the closing time	Linoleum	Brickwork Glass	
G3	2005	Urban (residential area)	S	252	745	35	2 days	$G3S_1$ $G3S_2$	In the middle of the morning and afternoon At the closing time	Wood	Brickwork Glass	Mechanical
			Bb	514	1843	100	2 days	$G3Bb_1$ $G3Bb_2$	At the closing time	Wood	Brickwork Glass	

[a]S1 – Studio 1, S2 – Studio 2; Bb – Bodybuilding.
[b]Maximum capacity.

A Greywolf (IAQ 610, WolfSense Solutions, USA) was used to continuously monitor the comfort parameters (temperature, relative humidity and CO_2) inside the rooms during the sampling days, from the opening to closing of the gymnasiums.[23] Outdoor meteorological data were obtained from Aeroporto weather station located in the centre of Lisbon (38 °46'N, 9 °08'W), from which data are available online.[29] MAS-100 and Greywolf were calibrated according to fabricant specifications.

Microbial characterization

Fungal colonies were grouped by macroscopic colony characteristics (e.g. colour, shape and elevation). For fungal identification, microscopic mounts were performed using tease mount or Scotch tape mount and lactophenol cotton blue mount procedures.[30] Morphological identification was achieved through macro and microscopic characteristics as given by de Hoog et al.[31]

The obtained bacterial isolates were characterized based on their macroscopic traits (e.g. pigmentation, texture and shape), microscopic morphology (cellular morphology, and presence/absence of endospores) and biochemical characteristics (gram staining, catalase and oxidase activities). For the morphological characterization, bacteria were isolated in Tryptic-Soy Agar medium and incubated at 30 °C for 24 h. The isolates were grouped into morphological types based on their characteristics. The definition of the morphological types was based on the Bergey's Manual of Determinative Bacteriology.[32] The frequency of each morphological type was calculated based on the number of isolates obtained and on their characters.

National guidelines for bioaerosols

In Portugal, a recent legislation established new limit values for microbiological contamination in indoor environments,[33] replacing the previous diploma.[34] In the previous legislation, a critical limit of 500 $CFU.m^{-3}$ was defined as the threshold for bacteria and fungi concentrations. Currently, the legal compliance is different concerning the type of microorganism. For fungi, indoor concentrations should be less than outdoor concentrations; and for bacteria, the indoor concentration should not exceed the outdoor concentration by 350 $CFU.m^{-3}$. However, when these situations are not fulfilled, there is a second opportunity to satisfy the legal requirements according to Tables 2 and 3.

The critical limit of 500 $CFU.m^{-3}$ was applied in guidelines and other studies.[33–37] In this present study, the sampling campaigns were performed when the previous legislation was in force, therefore the previous legislation limit was used to determine the legal

Table 2. Portuguese legal compliance for microbiological parameters according to Portaria no. 353-A/2013.

	Fungi	Bacteria
1st requirement	• [indoor] < [outdoor]	• [indoor] + 350 CFU. m^{-3} < [outdoor]
2nd requirement (to be applied when the 1st requirement is not fulfilled)	• No visible fungal growth on surfaces; • Species should be evaluated according Table 3	• [indoor] + 350 CFU. m^{-3} > [outdoor] and [CO_2] < 1800 mg.m^{-3}; • Ratio between Gram-negative bacteria and total bacteria should be less than 0.5.

Table 3. Fungal conformity based on the species according to Portaria no. 353-A/2013.

Species		Specific condition of conformity
Common species	*Cladosporium spp* *Penicillium spp* *Aspergillus spp* *Alternaria spp* *Eurotium spp* *Paecilomyces spp* *Wallemia spp..*	Mixture of species: ≤500 CFU.m^{-3}
Non-common species	*Acremonium spp* *Chrysonilia spp* *Tricothecium spp* *Curvularia spp* *Nigrospora spp*	One specie: <50 CFU.m^{-3} Mixture of species: <150 CFU.m^{-3}
Pathogenic species	*Chryptococcus neoformans* *Histoplasma capsulatum* *Blastomyces dermatitidis* *Coccidioides immitis*	Absence of any species
Toxigenic species	*Stachybotrys chartarum* *Aspergillus versicolor* *Aspergillus flavus* *Aspergillus ochraceus* *Aspergillus terreus* *Aspergillus fumigatus* *Fusarium moniliforme* *Fusarium culmorum* *Trichoderma viride*	One specie: <12 CFU.m^{-3} *(Several colonies per plate)*

compliance; and we also consider the compliance with the new limit requirements.

Statistical analysis

The Origin 7.5® software was used to compute graphical figures and the Statistica® software was used to calculate the statistical tests.

Results and discussion

Comfort parameters

According to the comfort criteria defined by the ISO 7730:2005,[38] the temperature should range between 23 °C and 26 °C and the relative humidity should vary between 30% and 70%. Table 4 presents the temperature and relative humidity measured in the three fitness centres during the sampling campaigns. Temperature varied between 10 °C and 27 °C with the greatest humidity levels recorded/observed in G1 (80%), exceeding the comfort criteria defined by ISO 7730:2005. The highest values for these parameters were recorded during occupancy of the spaces.[38]

CO_2 concentration was used not only as an indicator of ventilation efficiency, comfort and excess of occupancy but also to evaluate the microbiological compliance according to the Portuguese legislation. Table 4 shows the variation of indoor CO_2 concentrations measured during the sampling campaigns. CO_2 varied

Table 4. Physical parameters measured outdoor and indoor (temperature and relative humidity) and indoor CO_2 measured in fitness centres.

		Outdoor		Indoor		
Fitness centre	Sampling site	Temperature (°C) $\bar{x} \pm \sigma$	Relative humidity (%RH) $\bar{x} \pm \sigma$	Temperature (°C) $\bar{x} \pm \sigma$	Relative humidity (%RH) $\bar{x} \pm \sigma$	CO_2 (mg.m^{-3}) $\bar{x} \pm \sigma$
G1	Studio 1	19 ± 3.2	70 ± 15	18 ± 0.88	80 ± 4.1	1147 ± 502
	Studio 2			19 ± 1.01	78 ± 5.2	1315 ± 591
	Bodybuilding			19 ± 0.35	72 ± 2.6	1882 ± 553
G2	Studio	17 ± 2.4	61 ± 8.2	23 ± 1.7	58 ± 7.07	1185 ± 587
	Bodybuilding			21 ± 1.3	59 ± 7.8	1015 ± 219
G3	Studio	9.6 ± 2.3	64 ± 11	20 ± 1.1	69 ± 5.2	1122 ± 289
	Bodybuilding			20 ± 1.1	57 ± 5.6	1456 ± 355

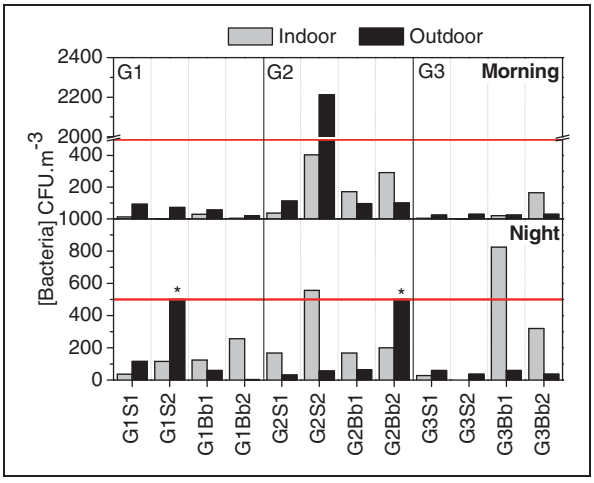

Figure 1. Concentrations of airborne bacteria measured indoors and outdoors of studied fitness centres. Results provided for each sampling site and sampling period. The horizontal line indicates the critical limit of 500 CFU.m^{-3}. The * indicates that the number of colonies were countless and therefore a concentration above 500 CFU.m^{-3} was assumed.

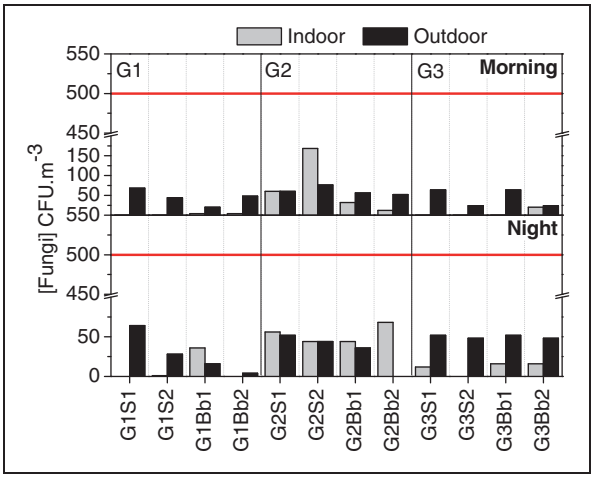

Figure 2. Concentrations of airborne fungi measured indoors and outdoors of studied fitness centres. Results provided for each sampling site and sampling period. The horizontal line indicates the critical limit of 500 CFU.m^{-3}.

between 398 mg.m^{-3} and 3590 mg.m^{-3} and showed a strong correlation between high occupancy and HVAC systems.[23] Higher CO_2 concentrations were observed during periods of physical activities within the studios.

Total bacteria and fungi concentrations

Figures 1 and 2 illustrate the indoor and outdoor concentrations of bacteria and fungi in the fitness centres.

Bacterial concentrations exceed the outdoor concentrations by 350 CFU.m^{-3} during the night period in the studio of G2 in the second day of sampling (556 CFU.m^{-3} indoor and 56 CFU.m^{-3} outdoor) and in the bodybuilding room of G3 in the first day of sampling (824 CFU.m^{-3} indoor and 60 CFU.m^{-3} outdoor). In the above situations, the critical limit of 500 CFU.m^{-3} was also exceeded. Results showed that at the end of the day, the bacterial load was significantly higher indoors than outdoors, indicating the effect of occupants on bacterial development.

For fungal concentrations, indoor concentrations were greater than outdoor concentrations in G2 in six measurements (sampling performed in the studio during the morning period and in all sampling performed at the end of the day) and in G1 in the bodybuilding room at night. Regarding the old guidelines,[33] the critical limit value of 500 CFU.m^{-3} was not exceeded in any situation. Results show that the highest

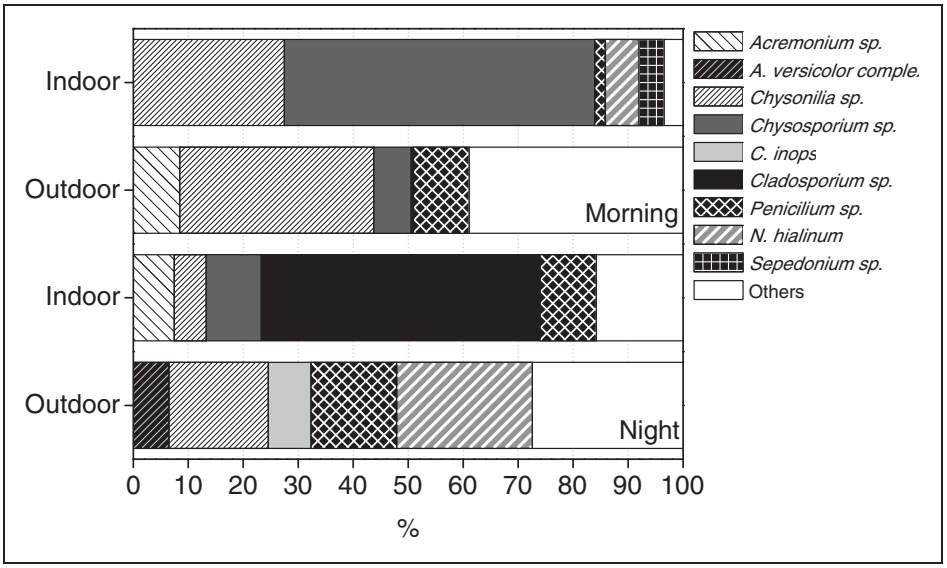

Figure 3. Frequency of the five most prevalent fungal genera in the two periods of sampling (morning and night), both indoors and outdoors.

concentrations were registered in G2 where there was natural ventilation, a phenomena which was also observed by Frankel et al.[39] In G1 and G3, outdoor particles were retained in the filters that were placed in the Air Treatment Units in both buildings, whereas in G2, outdoor air enters through the window spaces without any filtration. Moreover, G1 and G3 have mechanical ventilation, which is more efficient in promoting pollutant dilution.[40]

In general, there was an increase in bacterial load at night that was not observed with fungi. This suggests that the bacteria are more associated with human occupancy than fungi. The presence of bacteria indoors might be associated with deposited dust,[41] skin cells and hair.[42] These results are in agreement with that presented by Dacarro et al.[43] concerning the microbial load in universities and school gyms during physical education classes.

Identification of fungal species

The identification of fungal species is very important for the study of fungal contamination since it allows the differentiation between benign and harmful species.[31,44,45]

As presented in Figure 3, indoors, *Chrysosporium* sp. represented 56% of the fungal genera found in the air sample in the morning, the presence was reduced to 10% in the air sample taken at night, while 51% of the night time sample was identified as *Cladosporium* sp. The prevalence of *Chrysonilia* sp. was reduced from 27% in the morning to 5.8% before the closing time of the gym, and same behaviour was found outdoors, reducing the prevalence from 35% to 18%. The indoor *Penicillium* sp. was found to have increased from 1.9% to 10% between the two studied periods; this increase was also found in the outdoor samples (10% to 16%). *Acremonium* sp. was only identified indoors at the end of the day (7.5%). In fact, the most prevalent fungal genera found by our study are also consistent with other studies. *Cladosporium* sp. was widely found as the dominant genera inside buildings in many work.[46–50] Regarding sports facilities, a study conducted in a sports hall in China indicated that the dominant genera indoors were *Cladosporium* sp., *Penicillium* sp., *Aspergillus* sp. and *Alternaria* sp., making up 95% of the total observed genera.[51] Viegas et al.[24] described *Cladosporium* sp. as the principal isolated genera in a gymnasium, followed by *Penicillium* sp., *Aspergillus* sp., *Mucor* sp., *Phoma* sp. and *Crysonilia* sp. In a study conducted in houses in Barcelona, the greatest indoor concentrations of *Cladosporium* sp. was found during autumn;[52] the same trend was also found in infant bedrooms in the USA.[53] Species of *Cladosporium* sp. are widely distributed, commonly encountered on all kinds of plants and on debris and are frequently isolated from soil, food, paint, textiles and other organic matter,[54] therefore justifying the high prevalence of this fungi indoors at the end of the day because of the passage of debris from outdoors to indoors by people throughout the day.

A total of 22 genera and 27 species of fungal colonies were identified by this study. Table 5 shows the fungal species found indoors and outdoors of the fitness centre, during the two periods of sampling. Significant statistical differences were found in relation to the indoor and outdoor concentrations of fungal species,

Table 5. Distribution of fungal species indoor and outdoor in the two periods of sampling (morning and night). In bold are the five most prevalent fungal species identified in the morning (M) and at night (N), both indoors and outdoors.

	G1				G2				G3			
	Indoor		Outdoor		Indoor		Outdoor		Indoor		Outdoor	
Colonies	M	N	M	N	M	N	M	N	M	N	M	N
A. flavus complex								4				
A. fumigatus complex	4		11	4							8	8
A. niger complex			4				4					
Circumdati complex												8
A. ustus complex										4		
A. versicolor complex				20								
Acremonium sp.						**36**	20	8			40	
Alternaria sp.								24		4	16	
Aureobasidium sp.				12						4	16	8
Botrytis sp.							4					
Chrysonilia sp.			**35**	**12**	**72**	**28**	**56**	**32**			**24**	**24**
Chrysosporium sp.			**4**		**148**	**48**	28					4
C.inops			4	16				8				
Cladosporium sp.			8			**244**						4
Eurotium sp.							4					
Fusarium poae							8					
Geotrichum sp.				16		12	8			4		
Mucor sp.				4		8		16				
Neoscytalidium sp.						8	12					
N. dimidiatum						4	4					
N. hialinum				8	16	4	**28**	**32**		**8**		**56**
Paecilomyces sp.											24	
Penicillium sp.	**4**	**9**			**4**	**24**	**20**	**4**	**20**	**16**	**48**	**88**
Phoma sp.						4		4				
Rhodotorula sp.				4	4	8	12					
Scedosporium sp.			4				32					
Scopulariopsis sp.					4							
S. brevicaulis										4		
Sepedonium sp.					12							
Syncephalastrum racemosum					12							
Total	8	9	70	96	272	428	240	132	20	44	176	200

for G1 and G3 (Wilcoxon Matched Pairs test, G1, $p = 0.03$; G2, $p = 0.89$; G3, $p = 0.01$), although no significant statistical differences were found between the indoor concentrations among gymnasiums (Mann-Whitney test, $p = 1$ for all tests). In G1, two fungal species were identified (*Penicillium* sp. and one belonging to *A. fumigatus* complex), while in G2, 12 different genera and three species were found (*Acremonium* sp., *Chrysonilia* sp., *Chrysosporium* sp., *Cladosporium* sp., *Penicillium* sp., *Geotrichum* sp., *Mucor* sp., *Neoscytalidium* sp., *N. dimidiatum, N. hialinum, Rhodotorula* sp., *Sepedonium* sp., *Syncephalastrum recemosum, Scopulariopsis* sp., *Phoma* sp.). In G3, seven species were identified (one belonging to *A. ustus* complex, *Alternaria* sp., *Aureobasidium* sp., *Geotrichum* sp., *Penicillium* sp., *S. brevicaulis, N. hialinum*). As observed, the fungal load in G2 was higher than in other fitness centres. This can be explained by the fact that in G1 and G3, the fungi that come from outdoors are retained in the filters, whereas in G2, outdoor air enters in the rooms by the windows without any filtration. As emphasized in the studies of Frankel

Table 6. Frequencies of the isolated morphological groups (%).

	G1		G2		G3	
Morphological type	I	O	I	O	I	O
Gram-positive, catalase-positive cocci	3.2	5.1	0.11		1.7	
Gram-negative, catalase-positive cocci	25	58	30	55	30	38
Gram-negative, catalase-negative cocci	1.4				48	38
Non-spore forming, Gram-positive, catalase-positive bacilli	0.13	3.4				
Non-spore forming, Gram-positive, catalase-negative bacilli	2		0.16			6.3
Gram-negative, oxidase-positive bacilli		8.5	0.05	5.8		
Gram-negative, oxidase-negative bacilli	1.2	25		20	20	19

et al.[39] and Kemp et al.,[45] outdoor air is the main source of indoor fungi in healthy buildings.

Toxic species were found in G1 and G3 indoors, such as *Aspergillus* genus, belonging to *A. fumigatus* complex,[55–59] which is considered to be an indicator of moisture-damaged buildings.[60] Other most notable toxic mould belongs to *A. ustus* complex.

Fungal identification revealed one potentially dangerous situation (defined according Table 3) in the G2 studio associated with the presence of *Chrysonilia* sp. with a concentration of 72 $CFU.m^{-3}$. *Chrysonilia* sp. is considered a non-common species and is known to induce asthma.[61–63] In all the assessed gymnasiums, no sign of fungal growth was detected on the walls, furniture or in other materials.

Concerning the colonies concentration found indoor, there was a higher concentration found in the morning than at night that resulted in seven new species in G2 and six new species in G3. Some of these new isolates (*A. ustus* complex, *Acremonium* sp., *Alternaria* sp., *Aureobasidium* sp., *Cladosporium* sp., *Geotrichum* sp., *Mucor* sp., *Neoscytalidium* sp., *N. hialinum, Phoma* sp. *and S. brevicaulis*) can produce toxic compounds (metabolites or mycotoxins), though few of their metabolites have been shown to be produced in natural indoor environments.[8]

Bacteria characterization

Phenotypic characterization of the most prevalent isolates, collected by impaction in TSA medium, allowed for the identification of seven morphological groups, as summarized in Table 6.

Observing bacterial morphology and the Gram reaction usually constitutes the first stage of identification and is very useful for the preliminary identification of bacterial species. The traditional methods that employ observation of either single cell morphology or colony characteristics remain reliable parameters for the identification of bacterial species and still have significant taxonomic value.[64] Therefore, and despite being described by several authors as old fashioned, bacterial morphological characterization can provide valuable insights into individual microbial diversity, derived from both genetic and reversible changes.[65] Several morphotypes have been identified in bacteria related to chronic and acute infections, and specific phenotypic traits are important clinical features.[66–68]

According to the national legislation, when indoor concentrations exceed the outdoor concentrations by 350 $CFU.m^{-3}$, the ratio between the *Gram-negative* and the total bacteria should be less than 0.5. In the second day of sampling in G2 studio 2, the concentration of *Gram-negative* bacteria was calculated to be 540 $CFU.m^{-3}$ in a total of 556 $CFU.m^{-3}$, resulting in a ratio of 0.9. In G3 bodybuilding room 1, the concentration of *Gram-negative* bacteria was calculated to be 632 $CFU.m^{-3}$ in a total of 824 $CFU.m^{-3}$, giving a ratio of 0.7. Therefore, both locations failed to comply with the national legal compliance.

Our results have indicated that *Gram-negative, catalase-positive cocci* were the most prevalent airborne bacterial morphological-type indoors (25% in G1, 30% in G2 and 30% in G3) and outdoors (55% in G1, 30% in G2 and 38% in G3) of all fitness centres. In a study of cultivable airborne bacteria by the US Environmental Protection Agency in the Building Assessment Survey and Evaluation (BASE),[69] *Gram-negative cocci* were also found to be present within office buildings. The main source of *Gram-negative* bacteria is from settled dust,[70] brought into fitness centres by users, with the concentration of indoor particles affected by the levels of human occupancy.[41,71,72] Contamination can also be caused by outdoor particles due to the high prevalence of *Gram-negative cocci*. The second most prevalent bacterial phenotype was the *Gram-positive, catalase positive cocci*, appearing indoors in all the three studied gymnasiums. Several studies indicated that this phenotype is the most prevalent morphological type indoors.[28,70,73,74] This phenotype includes species such

as *Staphylococcus* and *Micrococcus*, which are abundant on human skin and on mucous membranes.[74,75] Our results were similar to those found by Bouillard et al.[70] in healthy office buildings once *Gram-positive catalase negative cocci* were not identified. G1 presented the highest morphological diversity when compared with the other fitness centres. As bacteria are strongly linked with levels of human occupancy, this result can be related to the higher occupancy of G1 and the need of more effective sanitation. This difference can be attributed to the higher levels of human occupancy within this gymnasium, as there is a strong correlation between human occupancy and bacterial diversity, revealing the need for more effective sanitation.

Conclusions

The indoor microflora is a complex mixture that varies according to the activities being undertaken, human occupancy levels, ventilation systems and physical parameters such as temperature and humidity. This work studied the microbiological load present in three fitness centres in the city of Lisbon, with results showing the existence of critical situations due to the presence of dangerous and toxic fungal species indoors. Natural ventilation used in G2 could have an influence on indoor fungal concentrations as no physical barrier exists to filter the outdoor air. For bacteria, nonconformities were recorded in G2 and G3. An increase in indoor bacterial concentration was observed during the evening that was not observed for fungal concentrations, thereby demonstrating the effect of human occupancy in the building on bacterial load.

Authors' contribution

All authors contributed equally in the preparation of this paper.

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References

1. Bünger J, Antlauf-Lammers M, Schulz T, Westphal G, Müller M, Ruhnau P and Hallier E. Health complaints and immunological markers of exposure to bioaerosols among biowaste collectors and compost workers. *Occup Environ Med* 2000; 57: 458–464.
2. Heldal K, Halstensen AS, Thorn J, Djupesland P, Wouters I, Eduard W and Halstensen TS. Upper airway inflammation in waste handlers exposed to bioaerosols. *Occup Environ Med* 2003; 60: 444–450.
3. Eduard W and Halstensen A. Quantitative exposure assessment of organic dust. *SJWEH Suppl* 2009; 7: 30–35.
4. Beuchat L, Komitopoulu E, Beckers H, Betts R, Bourdichon F, Fanning S, Joosten HM and Ter Kuile BH. Low-water activity foods: increased concern as vehicles of foodborne pathogens. *J Food Protect* 2013; 76(1): 150–172.
5. WHO. *WHO guidelines for indoor air quality: dampness and mould*. Copenhagen: World Health Organization (WHO), 2009.
6. Fang Z, Ouyang ZY, Hu LF, Wang X and Hu L. Culturable airborne bacteria in outdoor environments in Beijing, China. *Microb Ecol* 2007; 54: 487–496.
7. Shaffer BT and Lighthart B. Survey of airborne bacteria at four diverse locations in Oregon: urban, rural, forest and coastal. *Microb Ecol* 1997; 34: 167–177.
8. Jarvies B and Miller J. Mycotoxins as harmful indoor air contaminants. *Appl Microbiol Biot* 2005; 66: 367–372.
9. Carlisle A and Sharp N. Exercise and outdoor ambient air pollution. *Brit J Sport Med* 2001; 35(4): 214–222.
10. Canha N, Almeida SM, Freitas MC, Wolterbeek HT, Cardoso J, Pio CA and Caseiro A. Impact of wood burning on indoor $PM_{2.5}$ in a primary school in rural Portugal. *Atmos Environ* 2014; 94: 663–670.
11. Canha N, Almeida M, Freitas MC and Almeida SM. Seasonal variation of total particulate matter and children respiratory diseases at Lisbon basic schools using passive methods. *Proc Environ Sci* 2011; 4: 170–183.
12. Canha N, Almeida-Silva M, Freitas MC, Almeida SM and Wolterbeek HT. Lichens as biomonitors at indoor environments of primary schools. *J Radioanal Nucl Ch* 2012; 291(1): 123–128.
13. Canha N, Martinho M, Almeida-Silva M, Freitas MC, Almeida SM, Pegas PN, Alves C, Pio CA, Trancoso MA, Sousa R, Mouro F and Contreiras T. Indoor air quality in primary schools. *Int J Environ Pollut* 2012; 50(1-4): 396–410.
14. Canha N, Almeida SM, Freitas MC and Wolterbeek HT. Indoor and outdoor biomonitoring using lichens at urban and rural primary schools. *J Toxicol Env Heal A* 2014; 77: 14–16. (900-915.
15. Almeida SM, Canha N, Silva A, Freitas MC, Pegas PN and Alves C. Children exposure to air particulate matter in indoor of Lisbon primary schools. *Atmos Environ* 2011; 45(40): 7594–7599.
16. Almeida-Silva M, Almeida SM, Pegas PN, Nunes T, Alves CA and Wolterbeek HT. Exposure and dose assessment to particle components among an elderly population. *Atmos Environ* 2015; 102: 156–166.
17. Almeida-Silva M, Wolterbeek HT and Almeida SM. Elderly exposure to indoor air pollutants. *Atmos Environ* 2014; 8: 54–63.
18. Almeida-Silva M, Almeida SM, Gomes JF, Albuquerque PC and Wolterbeek HT. Determination of airborne nanoparticles in elderly care centers. *J Toxicol Env Heal A* 2014; 77(14–16): 867–878.
19. Viegas C, Almeida-Silva M, Gomes AQ, Wolterbeek HT and Almeida SM. Fungal contamination assessment in Portuguese elderly care centers. *J Toxicol Env Heal A* 2014; 77(1–3): 14–23.

20. Osman LM, Douglas JG, Garden C, Reglitz K, Lyon J, Gordon S and Ayres JG. Indoor air quality in homes of patients with chronic obstructive pulmonary disease. *Am J Resp Crit Care* 2007; 176: 465e72.
21. Bluyssen PM, Fernandes ED, Groes L, Clausen G, Fanger PO, Valbjorn O, Bernhard CA and Roulet CA. European indoor air quality audit project in 56 office buildings. *Indoor Air* 1996; 6: 221e38.
22. Almeida SM, Freitas MC, Reis M, Pinheiro T, Felix P and Pio CA. Fifteen years of nuclear techniques application to suspended particulate matter studies. *J Radioanal Nucl Ch* 2013; 297(3): 347–356.
23. Ramos CA, Almeida SM and Wolterbeek HT. Exposure to indoor air pollutants during physical activity in fitness centers. *Build Environ* 2014; 82: 349–360.
24. Viegas C, Alves C, Carolino E, Rosado L and Santos CS. Prevalence of fungi in indoor air with reference to gymnasiums with swimming pools. *Indoor Built Environ* 2010; 19(5): 555–561.
25. Viegas C, Alves C, Carolino E, Pinheiro C, Rosado L and Santos CS. Assessment of fungal contamination in a group of Lisbon's gymnasiums with a swimming pool. *Italian Assoc Industr Hygien* 2011; 2(1): 15–20.
26. Onchang R and Panyakapo M. The physical environments and microbiological contamination in three different fitness centres and the participants' expectations: Measurement and analysis. *Indoor Built Environ* 2014; 1420326X14543209.
27. Nunes I, Mesquita N, Cabo Verde S, Bandeira A, Carolino M, Portugal A and Botelho ML. Characterization of an airborne microbial community: a case study in the archive of the University of Coimbra, Portugal. *Int Biodeter Biodegr* 2013; 79: 36–41.
28. Li CS. Sampling performance of impactors for bacterial bioaerossols. *Aerosol Sci Tech* 1999; 30(3): 280–287.
29. Russia's Weather server. Weather Archive – World, Europe. Obtained from: http://meteo.infospace.ru/wcarch/html/index.sht (accessed 27 September 2014).
30. Fischer F and Cook NB. *Fundamentals of diagnostic mycology*. Philadelphia, PA: Saunders Company, 1998.
31. de Hoog C, Guarro J, Gené G and Figueiras M. *Atlas of clinical fungi*. 2nd ed. Ultrecht: Centraalbureau voor Schimmelcultures, 2000.
32. Holt JG. *Bergey's manual of determinative bacteriology*. 9th ed. Baltimore: Williams & Wilkins, 1994.
33. Portaria no. 353-A/2013. Requisitos de ventilação e qualidade do ar interior. Ministérios do Ambiente, Ordenamento do Território e Energia, da Saúde e da Solidariedade, Emprego e Segurança Social, Lisbon, 2013.
34. Decreto-Lei no. 79/2006. Regulamento dos Sistemas Energéticos de Climatização em Edifícios (RSECE). Ministério das Obras Públicas. Transportes e Comunicações, Lisbon, 2006.
35. ACGIH. *Guidelines for the assessment of bioaerosols in the indoor environment*. Cincinnati: American Conference of Governmental Industrial Hygienists, 1989.
36. Reynolds SJ, Streifel AJ and Mcjilton CE. Elevated airborne concentrations of fungi in residential and office environments. *Am Ind Hyg Assoc J* 1990; 51(71): 601–604.
37. The Government of Hong Kong. A guide on indoor air quality certification scheme for offices and public spaces, 2003: http://www.iaq.gov.hk/cert/doc/CertGuide-eng.pdf (accessed 15 March 2014).
38. ISO 7730:2005. *Ergonomics of the thermal environment — analytical determination and interpretation of thermal comfort using calculation of the PMV and PPD indices and local thermal comfort criteria*. Geneva: International Organization for Standardization, 2005.
39. Frankel M, Beko G, Timm M, Gustavsen S, Hansen E and Madsen A. Seasonal variations of indoor microbial exposures and their relation to temperature, relative humidity and air exchange rate. *Appl Environ Microb* 2012; 78(23): 8289–8297.
40. Canha N, Almeida SM, Freitas MC, Täubel M and Hänninen O. Winter ventilation rates at primary schools: comparison between Portugal and Finland. *J Toxicol Env Heal A* 2013; 76(6): 400–408.
41. Hospodsky D, Qian J, Nazaroff W, Yamamoto N, Bibby K, Rismani-Yazdi H and Peccia J. Human occupancy as a source of indoor airborne bacteria. *PLoS One* 2012; (4): 1–10.
42. Clark RP. Skin scales among airborne particles. *J Hygiene-Cambridge* 1973; 72: 47–51.
43. Dacarro C, Picco A, Grisoli P and Rodolfi M. Determination of aerial microbiological contamination in scholastic sports environments. *J Appl Microbiol* 2003; 95: 904–912.
44. Rao C, Burge H and Chang J. Review of quantitative standards and guidelines for fungi in indoor air. *J Air Waste Manage* 1996; 46: 899–908.
45. Kemp PC, Neumeister-Kemp HG, Murray F and Lysek G. Airborne fungi in non-problem buildings in a southern-hemisphere mediterranean climate: preliminary study of natural and mechanical ventilation. *Indoor Built Environ* 2002; 11(1): 44–53.
46. Jafta N, Batterman SA, Gqaleni N, Naidoo R and Robins TG. Characterization of allergens and airborne fungi in low and middle-income homes of primary school children in Durban, South Africa. *Am J Ind Med* 2012; 55: 1110–1121.
47. Baxi SN, Muilenberg ML, Rogers CA, Sheehan WJ, Gaffin J, Permaul P, Kopel LS, Lai PS, Lane JP, Bailey A, Petty CR, Fu C, Gold DR and Phipatanakul W. Exposures to molds in school classrooms of children with asthma. *Indoor Air* 2003; 13(1): 53–64.
48. Celtik C, Okten S, Aydogdu H, Bostancioglu M, Ekuklu G, Asan A and Yazicioglu M. Investigation of indoor molds and allergic diseases in public primary schools in Edirne city of Turkey. *Asian Pac J Allergy* 2011; 29(1): 42–49.
49. Soleimani Z, Goudarzi G, Naddafi K, Sadeghinejad B, Latifi SM, Parhizgari N, Alavi N, Babaei AA, Akhoond MR, Khaefi M, Rad HD, Mohammadi MJ and Shahsavani A. Determination of culturable indoor airborne fungi during normal and dust event days in Ahvaz, Iran. *Aerobiologia* 2013; 9(2): 279–290.
50. Oliveira M, Ribeiro H, Delgado JL and Abreu I. Aeromycological profile of indoor and outdoor environments. *J Environ Monitor* 2009; 11: 1360–1367.
51. Xie H, Gan XA and Ma F. Characteristics of bacterial and fungal aerosol in gymnasia in China. *Proc Int Conf Energy Environ Technol* 2009; 3: 19–23.
52. de Ana SG, Torres-Rodríguez JM, Ramírez EA, García SM and Belmonte-Soler J. Seasonal distribution of *Alternaria, Aspergillus, Cladosporium* and *Penicillium* species isolated in homes of fungal allergic patients. *J Invest Allerg Clin* 2006; 16(6): 357–363.
53. Ren P, Jankun TM, Belanger K, Bracken MB and Leaderer BP. The relation between fungal propagules in indoor air and home characteristics. *Allergy* 2001; 56(5): 419–424.
54. Bensch K, Braun U, Groenewald JZ and Crous PW. The genus *Cladosporium*. *Stud Mycol* 2012; 72: 1–401.
55. Land CJ, Sostaric B, Fuchs R, Lundstrom H and Hult K. Intratracheal exposure of rats to *Aspergillus fumigatus* spores isolated from sawmills in Sweden. *Appl Environ Microb* 1989; 55: 2856–2860.
56. Orciuolo E, Stanzani M, Canestraro M, Galimberti S, Carulli G, Lewis R, Petrini M and Komanduri KV. Effects of Aspergillus fumigatus gliotoxinand methylprednisolone on human

neutrophils: implications for the pathogenesis of invasive aspergillosis. *J Leukocyte Biol* 2007; 82: 839–848.
57. dos Santos VM, Dorner JW and Carreira F. Isolation and toxigenicity of Aspergillus fumigatus from moldy silage. *Mycopathologia* 2003; 156: 133–138.
58. El-Shanawany AA, Mostafa ME and Barakat A. Fungal populations and mycotoxins in silage in Assiut and Sohag governorates in Egypt, with a special reference to characteristic aspergilli toxins. *Mycopathologia* 2005; 159: 281–289.
59. Fischer G, Muller T, Ostrowski R and Dott W. Mycotoxins of *Aspergillus fumigatus* in pure culture and in native bioaerosols from compost facilities. *Chemosphere* 1999; 38: 1745–1755.
60. Samson RA, Flannigan B, Flannigan ME, Verhoeff AP, Adan OCG and Hoekstra ES. Air quality monographs. *Health implications of fungi in indoor air environments*. Vol 2, Amsterdam, The Netherlands: Elsevier Publications, 1994, pp.531–538.
61. Francuz B, Yera H, Geraut L, Bensefa-Colas L, Nghiem ZH and Choudat D. Occupational asthma induced by *Chrysonilia sitophila* in a worker exposed to coffee grounds. *Clin Vaccine Immunol* 2010; 17: 1645–1646.
62. Monzón S, Gil J, Ledesma A, Ferrer L, San Juan S and Abós T. Occupational asthma IgE mediated due to *Chrysonilia sitophila* in coffee industry. *Allergy* 2009; 64(11): 1686–1687.
63. Tarlo SM, Wai Y, Dolovich J and Summerbell R. Occupational asthma induced by *Chrysonilia sitophila* in the logging industry. *J Allergy Clin Immun* 1996; 97(6): 1409–1413.
64. Tshikhudo P, Nnzeru R, Ntushelo K and Mudau F. Bacterial species identification getting easier. *Afr J Biotechnol* 2013; 12(41): 5975–5982.
65. Sousa AM, Machado I, Nicolau A and Pereira MO. Improvements on colony morphology identification towards bacterial profiling. *J Microbiol Meth* 2013; 95: 327–335.
66. Proctor RA, von Eiff C, Kahl BC, Becker K, McNamara P, Herrmann M and Peters G. Small colony variants: a pathogenic form of bacteria that facilitates persistent and recurrent infections. *Nat Rev Microbiol* 2004; 4: 295–305.
67. Hogardt M and Heesemann J. Adaptation of *Pseudomonas aeruginosa* during persistence in the cystic fibrosis lung. *Int J Med Microbiol* 2000; 300: 557–562.
68. Hilmi D, Parcina M, Bode K, Ostrop J, Schuett S, Heeg K, Ziebuhr W, Sommerburg O and Bekeredjian-Ding I. Functional variation reflects intra-strain diversity of *Staphylococcus aureus* small colony variants in the host–pathogen interaction. *Int J Med Microbiol* 2013; 303: 61–69.
69. Tsai FC and Macher JM. Concentrations of airborne culturable bacteria in 100 large US office buildings from the BASE study. *Indoor Air* 2005; 15(9): 71–81.
70. Bouillard L, Michel O, Dramaix M and Devleeschouwer M. Bacterial contamination of indoor air, surfaces, and settled dust, and related dust endotoxin concentrations in healthy office buildings. *Ann Agr Env Med* 2005; 12: 187–192.
71. Canha N, Almeida SM, Freitas MC, Trancoso M, Sousa A, Mouro F and Wolterbeek HT. Particulate matter analysis in indoor environments of urban and rural primary schools using passive sampling methodology. *Atmos Environ* 2014; 83: 21.
72. Almeida-Silva M, Almeida SM and Wolterbeek HT. Multi-elemental characterization of indoor aerosols in elderly care centers. *J Radioanal Nucl Ch* 2014; 300(2): 679–684.
73. di Giulio M, Grande R, Di Campli E, Di Bartolomeo S and Cellini L. Indoor air quality in university environments. *Environ Monit Assess* 2010; 170: 509–517.
74. Aydogdu H, Asan A and Otkun MT. Indoor and outdoor bacteria in child day-care centers in Edirne cuty (Turkey), seasonal distribution and influence of meteorological factors. *Environ Monitor Assess* 2010; 164: 53–66.
75. Fox A, Harley W, Feigley C, Salzberg D, Toole C, Sebastian A and Larsson L. Large particles are responsible for elevated bacterial marker levels in school air upon occupation. *J Environ Monitor* 2005; 7: 450–456.