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Assessment and determinants of airborne bacterial and fungal concentrations in different indoor environments: Homes, child day-care centres, primary schools and elderly care centres

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HIGHLIGHTS

- The highest bacterial and fungal concentrations obtained in school and kindergartens.
- Occupancy and poor ventilation were associated with bacterial concentrations.
- *Penicillium* and *Cladosporium* were the most occurring fungi indoors.
- Children exhibited dose rates twice higher than adults.

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ABSTRACT

Until now the influence of risk factors resulting from exposure to biological agents in indoor air has been far less studied than outdoor pollution; therefore the uncertainty of health risks, and how to effectively prevent these, remains.

This study aimed (i) to quantify airborne cultivable bacterial and fungal concentrations in four different types of indoor environment as well as to identify the recovered fungi; (ii) to assess the impact of outdoor bacterial and fungal concentrations on indoor air; (iii) to investigate the influence of carbon dioxide (CO_2), temperature and relative humidity on bacterial and fungal concentrations; and (iv) to estimate bacterial and fungal dose rate for children (3–5 years old and 8–10 years old) in comparison with the elderly.

Air samples were collected in 68 homes, 9 child day-care centres, 20 primary schools and 22 elderly care centres, in a total of 264 rooms with a microbiological air sampler and using tryptic soy agar and malt extract agar culture media for bacteria and fungi growth, respectively. For each building, one outdoor representative location were identified and simultaneously studied.

The results showed that child day-care centres were the indoor microenvironment with the highest median bacterial and fungal concentrations (3870 CFU/m^3 and 415 CFU/m^3, respectively), whereas the lowest median concentrations were observed in elderly care centres (222 CFU/m^3 and 180 CFU/m^3, respectively). Indoor bacterial concentrations were significantly higher than outdoor concentrations ($p < 0.05$); whereas the indoor/outdoor ratios for the obtained fungal concentrations were approximately around the unit. Indoor CO_2 levels were associated with the bacterial concentration, probably due to occupancy and insufficient ventilation. *Penicillium* and *Cladosporium* were the most frequently occurring fungi. Children's had two times higher dose rate to biological pollutants when compared to adult individuals. Thus, due to children's susceptibility, special attention should be given to educational settings in order to guarantee their healthy future development.

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1. Introduction

Individual exposure to airborne bacteria and fungi has become a subject of concern over recent years due to the related adverse health effects. World Health Organization (WHO) has warned for health problems associated with building moisture and biological agents including respiratory symptoms, allergies and asthma as well as perturbation of the immunological system (Mendell et al., 2011).

Sources of airborne bacteria in built environments include the presence of humans, pets, soils, and plants (Bowers et al., 2012; Lignell, 2008; Womack et al., 2010). Outdoor air sources of bacteria can change over short periods of time related with climatic conditions (Rintala et al., 2008; Womack et al., 2010), however this influence on indoor air bacterial concentrations is less predominant. Kembel et al. (2012) indicated that natural ventilation does influence airborne bacterial concentrations in the absence of active human occupants, but we currently have little understanding of the relative influences of ventilation. Nor do we have an understanding of the relationship between indoor and outdoor airborne microbial concentrations.

Sources for indoor airborne fungi can be outdoor air and indoor reservoirs (WHO, 2009; Crawford et al., 2015). Fungal spores in outdoor air are a major source for indoor fungi during the growing seasons (e.g., spring and summer) for naturally ventilated buildings (WHO, 2009). Growth conditions like excessive humidity and/or a high water content of building materials are encountered on a more frequent basis, which in most cases can be described as the limiting factor for microbial growth. This is often caused by shortcomings in the buildings, such as lack of thermal insulation, as well as the incorrect behaviour of users of rooms (WHO, 2009). The complexity of bacterial and fungal indoor exposure (spatial variability, indoor sources, infiltrations from outdoor emissions, seasonal variability) indicates a need to further characterize their presence in different environments in order to understand the potential dose rates and consequently evaluate their impact on human health. Due to their ubiquitous presence in nature, the presence of biological agents is almost inevitable in most enclosed environments. This is especially relevant for susceptible groups such as children and elderly people which spend most of the time indoors (WHO, 2009).

Various studies on air quality and children's health indicate that indoor residential risk factors of primary interest for asthma, allergies, and respiratory health include biological agents (Bornehag et al., 2005; Cooley et al., 2004; Mendell, 2007). Besides their homes, children spend a large part of their time in schools and child day-care centres. The “Dampness in Buildings and Health” study, in Sweden (a cross-sectional postal questionnaire replied by parents of 10851 children, aged 1–6 years) found that attending day-care centres was associated with an increased risk of symptoms related to infections of airways and eczema (Hagerhed-Engman et al., 2006). In this large study, building ventilation systems, dampness and mould problems, as well as other building related factors, were suggested as probable causes.

Elderly people may also be particularly at risk of detrimental effects from air pollutants due to their reduced immunological defenses and multiple underlying chronic diseases (United Nations, 2012). The age of the European population is rising and the percentage of adults older than 65 years will increase from 16% in 2000 to 20% in 2020 (United Nations, 2012). Therefore, the assessment of biological air contamination in elderly care centres also raises a special concern.

Information about the concentrations and distribution of airborne bacteria and fungi is essential for conducting appropriate intervention intended to protect susceptible populations from being exposed to a hazardous indoor environment; particularly because these information is scarce and loosely in Portugal (Pegas et al., 2011; Madureira et al., 2014; Viegas et al., 2014).

The aim of the current work was to study airborne bacterial and fungal concentrations in different Portuguese indoor environments (homes, child day-care centres, primary schools and elderly care centres). The specific objectives of this work were: (i) to quantify airborne cultivable bacterial and fungal concentrations which could be potentially harmful to human health in four different indoor environments as well as to identify the recovered fungi; (ii) to assess the impact of outdoor bacterial and fungal concentrations on indoor air; (iii) to investigate the influence of carbon dioxide (CO_2), temperature and relative humidity on bacterial and fungal concentrations; and (iv) to estimate dose rates of bacterial and fungal for 3–5 and 8–10 years old children, in comparison with the elderly.

2. Materials and methods

Due to budget and time limitations the current work investigated 68 homes (families with children aged 8–10 years old), 9 (out of 40) child day-care centres (children aged 3–5 years old); 20 (out of 53) primary schools (children aged 8–10 years old) and 22 (out of 58) elderly care centres (adults aged over 65 years old) located in urban area of Porto city, North of Portugal (41 °N, 8W), featuring a Mediterranean climate with moderate temperatures and rainy weather in the winter season.

Two to eight rooms in each building (depending on the size) were randomly selected and investigated in a total of 264 rooms (68 bedrooms in homes; 50 classrooms in child day care centres; 73 classrooms in primary schools, and 73 bedrooms in elderly care centres). The walkthrough survey and the air sampling in each building occur within the same visitation period. The field work was conducted during winter seasons, from November to March, during years 2011–2013.

2.1. Walkthrough survey and checklist

A walkthrough survey was completed for each building and individual rooms to gather information on building structure, age and size, number of floors, finishing materials and floors conditions; walls; ceilings and operable exterior doors and windows; past occurrences and visible problems related to moulds and water; heating and ventilation systems and processes to maintain and operate the building and its activities (e.g., cleaning activities/schedule, renovation and retrofitting activities) and number of occupants.

The main characteristics of the investigated buildings and rooms are summarized in Table 1.

2.2. Air sample collection

Indoor samples were collected near occupants' breathing zone (approximately 0.7–1.5 m above the floor). Sampling locations were no closer than 1 m to a wall, window, door, or an active heating system. For each building, one outdoor representative location were identified and simultaneously studied, whenever possible no closer than 1 m from the building at heights of 1–2 m above the ground. The air sample collection included daytime sampling starting at 10 a.m. and was conducted discretely to minimize nuisance to normal occupant activities.

Bacterial and fungal air samples were obtained using a single-stage microbiological air impactor (Merck Air Sampler MAS-100), according to NIOSH method 0800 (1998) and EN 13098 (EN, 2000). Tryptic Soy Agar (TSA) (supplemented with 0.25% cycloheximide) and Malt Extract Agar (MEA) (supplemented with 1% of

Table 1
Characteristics of the buildings and rooms.

	Homes	Child day-care centres	Primary schools	Elderly care centres
	n (%)	*n* (%)	*n* (%)	*n* (%)
Buildings[a]	68	9	20	22
Construction period[b]				
Before 1980	14 (29)	1 (11)	17 (85)	11 (79)
1980–1989	3 (6)	3 (33)	2 (10)	0
1990–1999	14 (29)	3 (33)	1 (5)	2 (14)
2000–2009	18 (36)	2 (23)	0	1 (7)
Rooms[a]	68	50	73	73
Typology	Bedrooms	Classrooms	Classrooms	Bedrooms
Surface area and occupancy				
Floor area (m^2)[c]	12 (3)	36 (12)	51 (6)	19 (7)[d]
Occupants per room (no.)[c]	1 (0.6)	17 (5)	21 (3)	2.7 (1.5)[d]
Density of occupation (m^2/occupant)[c]	9.1 (3.5)	2 (2.3)	2.4 (0.4)	6.9 (4.6)
Type of floor				
Synthetic smooth (PVC/vinyl, linoleum)	2 (3)	48 (96)	48 (66)	35 (48)
Laminate parquetry	53 (80)	0	25 (34)	0
Wood/Cork	6 (9)	2 (4)	0	15 (20)
Stone/ceramic tiles	5 (8)	0	0	23 (32)
Heating system				
Hot water radiators/convectors	13 (30)	4 (44)	9 (12)	12 (53)
Electrical radiators/convectors	3 (7)	5 (56)	60 (82)	9 (43)
Heating floor	0	0	4 (5.5)	0
Other	27 (63)	0	0	1 (4)
Ventilation				
Natural	68 (100)	8 (89)	73 (100)	3 (14)
Mechanical	0	1 (11)	0	0
Natural and mechanical	0	0	0	19 (86)
Damp stains[e]	16 (24)	4 (44)[1]	14 (19)	13 (61)
Visible mould	13 (20)	N.A.	20 (27)	N.A.
Observations of condensation on the windows	34 (53)	4 (44)	36 (49)	13 (61)

[a] Number of buildings or rooms surveyed.
[b] Denominator for each variable may vary due to missing data.
[c] Mean (standard deviation); N.A.: not available.
[d] Correspond to 26 bedrooms.
[e] Infiltrations in classrooms.

chloramphenicol) were used as culture media for bacteria and fungi, respectively. Air was drawn through the sampler at a 100 L/min rate and sequential duplicate air samples of 100 and 250 L were collected both indoors and outdoors. The volume (and consequently the duration) of sequential air sampling was the same in all buildings and within all rooms in a specific building. For each sampling day, one field blank, per culture medium, was used. The air sampler was always cleaned between sample collections with cotton wipes wetted with isopropyl alcohol. After sampling, the agar media plates were sealed, marked and transported to the laboratory in a thermal bag for incubation.

2.3. Microbial analysis

To quantify the bacterial and fungal concentrations samples were incubated at 37 ± 1 °C for 48 ± 3 h and at 25 ± 3 °C for 72 ± 3 h, respectively (EN 13098, 2000). Quantification of bacteria and fungi levels was performed by naked eye count in accordance to the methodologies expressed in ISO 4833: 2013 (ISO, 2013) and EN 13098: 2000 (2000). The number of colonies recovered on the air-sample plates was adjusted using a positive-hole correction factor, and the results were expressed as number of colony forming units per cubic metre of air (CFU/m^3). The correction factor was based on the Fellers law (Andersen, 1958). The quantification limit is established as 10 CFU per plate.

Fungal identification was performed after 7 days of incubation, either on the original sampling media-MEA plates or after subculturing procedures, whenever colony isolation and growth observation were needed. Subculture was made on MEA plates and incubated, at 25 ± 3 °C, for periods ranging from 3 days to 3 weeks. Identification of fungal colonies was based upon phenotypic characteristics and followed standard mycological procedures based on their micro and macro-morphological characteristics (Atlas and Bartha, 1981).

The microbial analysis were performed by the Environmental Health Department of the National Health Institute using methodologies accredited by NP EN ISO/IEC 17025:2005, "General requirements for the competence of testing and calibration laboratories" (2005).

2.4. Carbon dioxide, temperature and relative humidity measurements

Carbon dioxide concentrations, temperature and relative humidity levels were continuously measured for a period of 7 days at homes and 5 days in child day-care, primary schools and elderly care, at 5-min intervals, using a portable indoor air quality metre IAQ-CALC (model 7545; TSI, Inc.). Within each building these measurements were recorded concurrently. The instruments were calibrated once per year according to manufacturer specifications.

2.5. Dose rate

Dose rates were calculated using Equation (1), which was validated in previously published studies (Castro et al., 2011; Fonseca et al., 2014; Kalaiarasan et al., 2009):

$$D = \left(\frac{BR_{WA}}{BW}\right) \times C_{WA} \times OF \times N \quad (1)$$

In this equation, D represents the age-specific dose rate (CFU/kg/day); BR_{WA} is the age-specific weighted average breathing rate (L/min); BW is the body weight of the children/elderly (kg); C_{WA} is the weighted average bacteria or fungi concentrations (CFU/L); OF is the occupancy factor; N is the total time per day spent in the location of exposure (min/day). The main daily activity patterns (including residence time and type of performed activities) of the children and elderly people were registered and analysed. The BR_{WA} is characterized by the intensity of the activity practiced at the time of exposure. Since in the current study children and elderly people spend their time having a nap, sleeping or seated normally (e.g. writing, reading, watching TV, drawing), the "sedentary/passive" activity level was selected. The age-specific inhalation factors (male and female combined) were retrieved from the US EPA exposure factors handbook (U. S. Environmental Protection Agency, 2011) since there is no available information concerning the Portuguese population. Thus, BR_{WA} was considered as 4.5 L/min in sleep or nap; and 4.8 L/min for sedentary activities for 8 to 10-year-old children. BR_{WA} values of 5.2 L/min and 4.9 L/min were used for elderly people for sleep or nap and sedentary activities, respectively. Regarding the children attending the child care centres (ages 3–5 years old), the BR_{WA} corresponds to 4.8 L/min. C_{WA} was estimated using the bacteria and fungi median concentrations and the OF was always considered as 1, since both children and elderly kept their schedules and their respective locations tightly. At child care centres and primary schools (3–5 and 8–10 years old) children had similar daily schedules and/or activity patterns, spending about 6 h indoors. For homes and elderly care centres the time was divided into

Table 2
Descriptive statistics of indoor and outdoor concentrations of carbon dioxide, temperature, relative humidity, bacteria and fungi and I/O ratios of bacteria and fungi concentrations.

	Homes		Child day-care centres		Primary schools		Elderly care centres		
	Median (P25–P75)	Range	Median (P25–P75)	Range	Median (P25–P75)	Range	Median (P25–P75)	Range	*p*-value
Carbon dioxide (ppm)									
Indoor	1107 (815–1458)	509–2641	1506 (790–1946)	382–2708	1469 (1195–2104)	829–3111	1033 (760–1268)	579–2697	<0.05
Outdoor	490 (405–578)	318–853	365 (338–412)	329–559	442 (364–504)	349–636	580 (552–640)	516–879	<0.05
Temperature (°C)									
Indoor	16.9 (15.5–18.3)	10.7–23.0	18.7 (17.5–20.8)	13.6–25.9	20.8 (19.2–21.7)	14.3–24.6	20.0 (18.0–22.0)	13.0–27.0	<0.05
Outdoor	13.2 (12.1–14.5)	8.1–19.2	18.3 (15.1–21.2)	11.4–33.4	14.5 (11.7–16.9)	10.0–20.6	17.0 (16.0–19.2)	11.0–23.0	<0.05
Relative humidity (%)									
Indoor	66 (59–71)	36–84	57 (50–66)	28–83	54 (50–65)	34–74	52 (37–62)	24–73	<0.05
Outdoor	73 (66–80)	40–92	49 (31–57)	19.66	59 (53–68)	40–75	50 (33–66)	18–80	<0.05
Bacteria (CFU/m^3)									
Indoor	684 (350–1618)	98–6528	3870 (1498–5705)	190–52560	3224 (1784–5430)	168–8372	222 (20–338)	20–630	<0.05
Outdoor	278 (77–1006)[a]	10–6528	120 (72–122)	36–146	213 (79–853)	20–3684	89 (30–86)	80–368	<0.05
Fungi (CFU/m^3)									
Indoor	250 (119–566)	34–6528	415 (280–711)	60–38580	240 (169–400)	61–1322	180 (108–306)	18–1218	0.005
Outdoor	184 (116–553)[a]	18–6528	241 (163–330)	152–335	200 (112–302)	53–590	174 (96–238)	62–676	<0.05
Indoor/outdoor ratio bacteria[b]	3.72 (0.46–10.2)	0.05–99.0	40.2 (26.5–124.7)	11.8–141.5	8.82 (3.94–37.2)	0.82–119.4	4.81 (1.66–8.98)	0.46–30.4	<0.05
Indoor/outdoor ratio fungi[b]	1.24 (0.38–3.56)	0.01–139.3	2.36 (1.77–5.42)	0.68–67.6	1.26 (0.87–2.56)	0.50–4.23	1.26 (0.94–1.41)	0.57–3.11	<0.05

Homes: n (indoor) = 68; n (outdoor) = 43.
[a] n (outdoor) = 68; Child day-care centres: n (indoor) = 50; n (outdoor) = 9; Primary schools: n (indoor) = 73; n (outdoor) = 20; Elderly care centres: n (indoor) = 72; n (outdoor) = 22; P25–P75: 25th-75th percentile.
[b] Correspond to the number of indoor measurements matched with the correspondent outdoor measurements.

two different main activity levels, sleep or nap (10 h for both age groups) and sedentary levels (around 14 h for the children and the elderly). Body weight of 18.6 kg for 3–5-year old children; BW of 35 kg for 8-10 year-old for children and BW of 77 kg for adults (>65 years old) were used (U. S. Environmental Protection Agency, 2011). The dose rates were estimated using the bacteria and fungi average concentrations (weighted by the real time that children or elderly spent in each indoor environment).

2.6. Statistical analysis

SPSS (version 19) software was used for all statistical analysis. Shapiro–Wilk test was used for normality testing. The distributions was skewed; thus they were described by median, 25th percentile (P25) and 75th percentile (P75). Kruskal–Wallis test was used to compare continuous variables. The indoor to outdoor (I/O) ratio was calculated to determine the impact of outdoor sources on indoor air concentrations. Spearman's rank correlation coefficient was also calculated to assess the influence of CO_2, temperature and relative humidity measurements on bacteria and fungi concentrations. Statistical significance was defined as $p < 0.05$.

3. Results

A summary of the environmental parameters is given in Table 2.

3.1. Bacterial and fungal concentrations

Table 2 presents the bacterial and fungal concentrations measured in the four different indoor settings. Bacterial concentrations varied on a large scale within and between the four studied building types. As a result and comparing the four indoor environments, the median concentration of bacteria was highest in child day-care centres and lowest in elderly care facilities. At child day-care centres and primary schools the median indoor concentrations of bacteria were approximately 17% and 14% higher than elderly care centres, respectively.

Regarding fungi, and comparing the four building types, the highest median fungal concentrations were measured in child day-care centres. The lowest median fungi concentrations were observed in elderly care centres.

3.2. Indoor/outdoor relationship

Table 2 presents the I/O ratio for each indoor environment. For all indoor environments, the median I/O ratios for bacteria concentrations were higher than 1, ranging between 3.72 and 40.2. The highest I/O ratios for bacteria concentrations were observed in child day-care centres and primary schools.

The median I/O ratio for fungi were 1.24 in homes, 2.36 in child day-care centres, 1.26 in primary schools, and 1.26 in elderly care centres, and the differences among the four different building types were statistically significant ($p < 0.05$).

3.3. Influence of CO_2, temperature and relative humidity

The correlation coefficients (r_s) between CO_2, temperature and relative humidity and bacterial and fungal concentrations are presented in Table 3.

There was a weak positive correlation between CO_2 levels and bacterial concentrations in child day-care centres. Similar finding

Table 3
Spearman's rank correlation coefficients.

	Homes	Child day-care centres	Primary schools	Elderly care centres
Carbon dioxide				
Indoor bacteria	0.01	**0.34**	**0.26**	0.19
Indoor fungi	−0.11	0.08	−0.17	−0.05
Temperature				
Indoor bacteria	0.16	−0.20	−0.17	0.08
Indoor fungi	0.02	0.20	0.14	**−0.29**
Relative humidity				
Indoor bacteria	−0.10	**0.47**	0.18	0.11
Indoor fungi	−0.04	−0.08	0.42	**0.37**

Significant correlations are marked in bold ($p < 0.05$).

was observed for the correlation between relative humidity and bacterial concentrations. In primary schools a weak positive correlation between CO_2 concentrations and bacterial concentrations was also found.

A weak negative correlation between indoor temperature and indoor fungal concentrations was found in elderly care centres, while a weak positive correlation was found for indoor relative humidity levels.

3.4. Cultivable airborne fungi identification

Table 4 shows the distribution of indoor and outdoor fungal genera/species identified. In all indoor environments, *Penicillium* and *Cladosporium* species were the most frequently detected fungi. Apart from *Penicillium* and *Cladosporium* species, other identified fungi genera included *Rhodotorula sp.*, *Aspergillus* (*Aspergillus fumigatus* and *Aspergillus niger*), *Fusarium sp.*, *Geotrichum sp.*, *Alternaria sp.* and yeast. Lower diversity of cultivable fungi genera/species was observed in elderly care centres when compared with the other indoor environments.

Outdoor airborne identified fungi were generally similar to the ones found indoor: *Penicillium sp.* was observed most frequently, followed by *Cladosporium sp.* Some fungal genera/species as *Alternaria sp.*, *Fusarium sp.*, *Aspergillus fumigates*, *Geotrichum sp.*, *Aspergillus sp.* and *Aureobasidium pullulans* were found more often outdoors.

3.5. Dose rates analysis

The inhalation dose rates of bacteria and fungi were estimated for 3–5-year-old children, for 8–10-year-old children and for the elderly (≥65 years old) (Table 5).

Chid day-care centres represent the worst scenario from the four studied environments, exhibiting the highest levels both for, bacteria and fungi doses rates (5.49×10^8 and 3.72×10^7, respectively). Regarding the bacteria dose rates, higher levels are observed in educational environments compared to the domestic environments studied. The dose rate was, in the group of children aged 8–10 years old, 7.88×10^7 CFU/kg/day in homes, being higher in primary schools (1.52×10^8 CFU/kg/day), whereas the dose rates of fungi for this age group were higher at homes than in primary schools. Children attending child day-care centres had higher bacterial dose rates than those attending primary schools. The similar kind of trend was observed for fungal dose rates.

Table 5
Age-specific dose rates (CFU/kg/day) of bacteria and fungi by indoor environment.

	Homes (8–10 yr)	Child day-care centres (3–5 yr)	Primary schools (8–10 yr)	Elderly care centres (≥65 yr)
Dose rate bacteria				
Sleep or nap	5.28×10^7	–	–	8.10×10^6
Sedentary	7.88×10^7	5.49×10^8	1.52×10^8	1.27×10^7
Dose rate fungi				
Sleep or nap	1.93×10^7	–	–	5.56×10^6
Sedentary	2.88×10^7	3.72×10^7	1.22×10^7	1.03×10^7

To further understand the dose to airborne bacteria and fungi at homes, child day-care centres and primary schools, the dose rates for children were compared with those for elderly people. The results showed that inhalation dose rates were lower in adults, being minimal when elderly are sleeping or in a nap (bacteria dose rate: 8.10×10^6 CFU/kg/day; fungi dose rate: 6.56×10^6 CFU/kg/day) (Table 5).

4. Discussion

4.1. Bacterial and fungal concentrations

There were significant differences between bacterial and fungal concentrations among the four different indoor environments investigated. In general, it was demonstrated that the indoor concentrations of airborne cultivable bacteria and fungi were higher in child day-care centres. These environments are critical for children as children are prone to infections and allergic reactions, since their immune system has not yet matured (WHO, 2009). In primary

Table 4
Distribution (percentage) of indoor and outdoor fungal genera/species.

Fungi genera/species	Indoor air				Outdoor air			
	Homes	Child day-care centres	Primary schools	Elderly care centres[a]	Homes	Child day-care centres	Primary schools	Elderly care centres[a]
Acremonium sp.	13.2	8.0	10.5	0	30.9	11.1	30.0	0
Alternaria sp.	26.5	20.0	66.7	0	47.1	77.8	75.0	0
Aspergillus sp.	17.6	26.0	25.0	0	16.2	44.4	20.0	0
Aspergillus flavus	7.4	6.0	0	0	4.4	11.1	0	0
Aspergillus fumigatus	58.8	4.0	45.0	1.4	63.2	22.2	40.0	0
Aspergillus nidulans	4.4	0	0	0	1.5	0	0	0
Aspergillus niger	17.6	6.0	27.3	2.8	8.8	0	10.0	0
Beauveria sp.	0	0	0	0	1.5	0	0	0
Cladosporium sp.	91.0	100	100	35.2	97.1	100	90.0	53.0
Chaetomium sp.	0	0	0	0	0	0	5.0	0
Fusarium sp.	44.1	12.0	15.4	0	61.8	33.3	60.0	0
Geotrichum sp.	36.8	0	26.3	0	75.0	0	35.0	0
Yeast	45.6	58.0	100	2.8	47.1	33.3	55.0	0
Mycelia Sterilia	2.9	12.0	10.5	0	14.7	33.3	20.0	0
Paecilomyces sp.	5.9	14.0	27.8	0	5.9	33.3	35.0	0
Penicilium sp.	100	86.0	100	52.1	97.1	88.9	100	48.3
Phoma sp.	4.4	8.0	11.1	0	17.6	22.2	15.0	0
Rhizopus sp.	7.4	10.0	13.3	1.4	1.5	33.3	10.5	0
Rhodotorula sp.	73.5	80.0	100	4.2	42.6	22.2	45.0	0
Ulocladium sp.	2.9	4.0	5.6	0	0	22.2	15.0	0
Trichoderma sp.	4.4	2.0	12.5	0	7.4	0	20.0	0
Aureobasidium pullulans	11.8	12.0	21.4	0	14.7	44.4	15.0	0
Verticillium sp.	11.8	0	5.3	0	45.6	0	10.5	32.5
Curvularia sp.	1.5	0	0	0	0	0	0	0

[a] Correspond to the main fungi genera identified.

schools similar results were obtained as those observed in child day-care centres for both biological agents; whilst in the elderly care centres the lowest median bacterial and fungal concentrations were exhibited. One possible cause for this observation may be the higher occupancy and active behavioural pattern/activity level of children in relatively small spaces. In fact, the educational settings had the highest occupancy levels of the four different buildings surveyed (Table 1). This assertion is also supported by a number of previous studies in which human occupancy was found to affect indoor microbial concentrations, as settled spores were re-suspended by human activities (e.g. walking and running in indoor sites) (Kim and Kim, 2007; Mentese et al., 2009); this effect is assumed to be more pronounced with younger children.

In addition these findings might also be due to poor ventilation taking into account that most of the schools are naturally ventilated (Table 1) and, associated with higher occupant densities, higher levels of CO_2 were obtained (Table 2). Another possible explanation is the environmental conditions surrounding the buildings, as plants and soil outdoors; as well as the influence of season (winter time) and climate.

More than half of homes (54.4%) and over 90% of the child day-care centres and primary schools had a median bacteria concentration above the current Portuguese established levels (*indoor < outdoor+350 CFU/m*^{3}) (Ordinance n.º 353-A/2013). For elderly care centres, however, only 16.9% were found to be higher than the levels established by Portuguese legislation (Ordinance n.º 353-A/2013).

The same trend was observed for fungi concentration distribution: child day-care centres showed the highest indoor median fungi concentrations; while elderly care centres were the indoor environment which exhibited the lowest median fungal levels. In general, 53.3% rooms had indoor fungi concentrations higher than outdoors (60.0% in homes; 81.8% in child day-care centres; 57.5% in primary schools and 47.1% in elderly care centres), noncompliant with Portuguese legislation ("*indoor < outdoor*") (Ordinance n.º 353-A/2013).

The indoor concentrations of bacteria and fungi obtained in the current work were higher than other studies conducted in similar kind of indoor environments, such as the work of Roda et al. (2011) in child day-care centres in Paris and in Turkey (Mentese et al., 2009). Other studies, conducted in Portugal (Pegas et al., 2011; Viegas et al., 2014), reported higher concentrations of fungi at child day-care centres and elderly care centres than the present work. Seasonal influences, meteorological conditions, specific indoor sources and laboratory methods/analyses and lack of standardization could account for some of these differences (Nevalainen, 2007; Viegas et al., 2014). In addition, different study design (sampling period, duration, sample size) could also contribute to these differences.

4.2. Impact of outdoor on indoor bacterial and fungal concentrations

The I/O ratio gives some indication on where the source of bioaerosol exists. If the I/O ratio is greater than 1, the source is more likely in the indoor environment. At all indoor environments the outdoor concentrations of bacteria were significantly lower than indoors ($p < 0.05$). The median I/O ratio for bacteria was above 2 for most indoor environments, which indicates that their major sources might be any activities carried out in such rooms; density of occupation, and/or, ventilation (windows are closed more often in winter and ventilation might be insufficient) (Kim and Kim, 2007; Mentese et al., 2009). Thus indoor sources had a greater effect than the inflow from the outside. The statistical test results showed that there was a significant difference between them ($p < 0.05$). Similar to the current work, the previously conducted study in Portuguese primary schools located in Porto has also pointed to lower concentrations of bacteria outdoors when compared with indoors, and that the occupancy, indoor activities, and/or indoors sources are determinant for the observed differences (Madureira et al., 2014).

The impact of indoor sources for fungi was less evident. The results show that I/O ratio was slightly above 1 in homes (I/O = 1.24), child day-care centres (I/O = 2.36), primary schools (I/O = 1.26) and elderly care centres (I/O = 1.26). Since the median I/O ratio for fungi is around 1 and the indoor level of fungi was relatively low, it can be pointed out that outdoor air is one of the main sources for fungi, suggesting that the overall impact of outdoor sources seem more relevant than the contribution of indoor sources. These findings are in accordance with literature (Mentese et al., 2009; WHO, 2009).

4.3. Influence of CO_2, temperature and relative humidity

The Spearman correlation analysis showed a positive correlation between bacteria and CO_2 concentrations. CO_2 is considered a surrogate measure of the rate of outside air supplied per occupant (Daisey et al., 2003). This positive correlation with the number of bacteria suggests not only that there is some interdependence between the number of occupants and the concentration of bacteria, but also ventilation issues namely insufficient air renewal rates in educational settings such as child day-care centres and primary schools. This information could be used as a parameter of an excess of occupants/m^2 or insufficient ventilation of the rooms.

Temperature and relative humidity did not influence indoor bacteria and fungi concentrations; this was probably due to the small variation levels of both parameters indoors, which cannot allow any observed association with biological pollutants.

4.4. Recovered fungi identification

The composition of bioaerosol indoors may be important, and thus different health effects may result from exposure to different fungal profiles. Although the characteristics of the investigated indoor environments were different from one another, the two most predominant fungi genera (*Penicillium* and *Cladosporium* species) observed in this work are considered as allergens (Ege et al., 2011). Similarly, Mentese et al. (2009) reported that *Penicillium sp.*, *Aspergillus sp.* and *Cladosporium sp.* were the dominant genera in dwellings. According to Kim and Kim (2007) *Penicillium sp.*, *Cladosporium sp.* and *Aspergillus sp.* were the dominant genera in elderly care centres.

Meanwhile, other fungi genera/species were also found indoors, such as *Rhodotorula sp*, *Aspergillus* (*A. fumigatus* and *A. niger*). The composition of the fungal genera in outdoor air was generally similar to that of the indoor air, which clearly identifies outdoor sources as the major contributor to indoor air fungal composition.

Penicillium sp., as other fungi, when growing produce volatile organic compounds as their metabolites and have been implicated in asthma symptoms in children (Araujo et al., 2008). *Cladosporium* and *Alternaria* might induce or exacerbate hypersensitivity reactions, including asthma (Ege et al., 2011). The presence of toxin-producing fungi like *A. fumigatus* indoors is very common also in the outdoor air and, may hence occur in indoor air in low concentrations quite frequently (Daisey et al., 2003; Madureira et al., 2014). *Aspergillus species* (*Aspergillus flavus* and *A. fumigatus*), are well-known, potentially life threatening, airborne contaminants when blown in through the windows of wards containing immunocompromised patients, like the elderly (Tang, 2009). Even in homes and schools, fungi and their spores may trigger

hypersensitivity reactions such as rhinitis, sinusitis, or asthma in healthy individuals (Tang, 2009).

The differences in fungi genera prevalence between studies and indoor environments could be attributed to geographic location, season, differences in culture procedures, different experimental and sampling approaches, relative humidity and building characteristics (Madureira et al., 2014; Sen and Asan, 2009).

Thus, it is important to determine the fungal genera/species in health risk assessment since not all fungi have the same health effects; some are potentially allergenic (*Penicillium*, *Cladosporium herbarum*, *Alternaria alternata*, *A. fumigatus*, etc.) and other might cause toxic effects through the production of mycotoxins (*A. flavus*, *Trichoderma* and *Fusarium*, etc.) (Bush et al., 2006).

4.5. Dose rate analysis

The bacteria dose rates in schoolchildren aged 8–10 years old was higher in primary schools than at homes. These results are associated with the increased concentrations of bacteria found in primary schools. In contrast, the fungi dose rate for this age group was higher in homes than in primary schools, which is also associated with the higher levels found in the surroundings of domestic environments.

As expected, the risk of exposure is decreased in the elderly when compared to children, being minimal when they are sleeping or napping. Due to children's vulnerability and low weight, they still showed higher doses rates, both for bacteria and fungi, than the elderly. Considering the high susceptibility of young children, these results demonstrate that educational settings (child day-care centres and primary schools) are important environments for children's overall exposure to biological pollutants. However, the bacterial and fungal air samples were collected during daytime and were assumed that the exposure concentration is the same during sleeping, which could likely not be the case.

Information regarding schoolchildren's and elderly's dose rates to bacteria and fungi are limited and, thus, the findings on dose rates obtained within this work are difficult to compare due to the different approaches and the distinct aspects that characterize the sampled microenvironments. Therefore, the dose rates estimated within this work could not be compared with other studies.

4.6. Limitations of the study

The present work discloses some limitations. One of the constraints was the assessment period (winter season), therefore the influence of seasonal differences should be explored. Another limitation is the short sampling periods which may introduce important variations between measurements resulting in poor reproducibility and weak consistency in comparisons. However, up to 250 micro-environmental measurements were carried out using a similar protocol during the air sampling as well as in laboratory analysis.

The current study was conducted by air sampling. As the health effects of biological parameters are mainly respiratory, air sampling is believed to be adequate to represent exposure. However, biological aerosols have been found to exhibit varying patterns in their release and spread into the air depending on several environmental factors. Air sampling alone may give an incorrect picture of the biological diversity actually present in a building (Andersen et al., 2011). In addition, it is known that bioaerosol concentrations vary strongly indoors with human occupancy and activity. Short-term sampling may not effectively represent the time-averaged conditions experienced by occupants. There are also by now very well known limitations in culture-based sampling methods for characterizing health-related bioaerosol composition and concentrations indoors. Currently, there are numerous sampling methods available to measure biological concentrations in the environment. Source sampling, which include methods such as swab, tape, bulk, and dust, is commonly used to identify indoor bacteria and fungi (Niemeier et al., 2006). Specific and focused approaches should be considered in further studies.

5. Conclusions

This work fills a gap providing information on the bacterial and fungal concentrations both indoors and outdoors in Portugal: their determinants in a total of 264 rooms from four different indoor environments. The results demonstrated that indoor bacterial and fungal concentrations were the highest in child day-care centres and primary schools, which could be explained by differences in density of occupation, occupant activities and inadequate ventilation. With the exception of homes, child day-care centres and primary schools median concentrations of the airborne bacteria exceeded the Portuguese legislation. Regarding indoor median concentrations of fungi, all different environments exceeded the Portuguese legislation.

The I/O ratios for the observed fungi concentrations were calculated as approximately around 1, and for bacteria concentrations, higher than 2. A significant difference was found between indoor and outdoor bacteria concentrations. In addition, it was found that the indoor CO_2 concentration was correlated with the concentration of bacteria, probably due to occupancy and insufficient ventilation.

Penicillium, and *Cladosporium* were the most frequently found genera in indoor air. The other identified genera included *Rhodotorula sp*, *Aspergillus* (*A. fumigatus* and *A. niger*). Some species of the genera *Aspergillus*, such as *A. fumigatus*, have allergenic, toxigenic, and infectious impacts on health, and thus the identification of species should be a concern for future epidemiological studies.

Child susceptibility to bacteria and fungi exposure was supported by this study. When compared to elderly individuals in similar conditions, children exhibited at least two times higher dose rates of bacteria and fungi.

Therefore, special attention should be given to the major sources of bacteria and fungi in educational settings. Further investigations regarding building characteristics and sources of biological pollutants would be important to provide information to the public health policies.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.atmosenv.2015.03.026.

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