Aerobiologia (2021) 37:139–153
https://doi.org/10.1007/s10453-020-09681-5

ORIGINAL PAPER

Assessment of indoor air quality in geriatric environments of southwestern Europe

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Received: 15 October 2019 / Accepted: 3 December 2020 / Published online: 1 January 2021

Abstract The objective of this study was to evaluate physical–chemical and microbial indicators of indoor air quality in three nursing houses (NHs) located in rural areas of the northeast of Portugal. The parameters were measured during two campaigns (winter and summer), twice a day, and in four distinct spaces for spatial variability assessment: dining room, living room, double bedroom and outdoor of the NHs. Physical–chemical indicators were assessed by using a Graywolf IQ 610 probe. Airborne microbial levels were evaluated by direct impaction to the culture media, and subsequently, the microorganisms were identified molecularly. Mean concentrations of physical–chemical (with the exception of total volatile organic compounds, TVOCs) and microbial indicators did not exceed the legal limits. Overall, in all NHs, the indoor-to-outdoor (I/O) concentration ratios of chemical and biological pollutants were ≤ 1 in the summer, while in the winter were > 1. *Bacillus*, *Micrococcus* and *Staphylococcus* were the dominant bacterial genera, and *Aspergillus*, *Cladosporium* and *Penicillium* were the dominant fungal genera. The diversity of species was higher in summer. The main results suggest that a good air quality prevails in all studied spaces, although conditions less desirable have been identified in winter, indicating the need to deepen the study of air quality in these places, since these are occupied by elderly people who are more susceptible to infections.

Keywords Nursing home · IAQ · Biocontaminant · Indoor/outdoor ratio · Carbon oxides · Shannon–Wiener index

1 Introduction

Indoor air quality (IAQ) is one of the main factors affecting health, well-being and productivity of people (Hayleeyesus and Manaye 2014). In general, poor IAQ is related to the presence of pollutants (biological and chemical) and inadequate physical environmental conditions (temperature and relative humidity), to which occupants of indoor environments are exposed (Hayleeyesus and Manaye 2014; Sattar and Bact 2016).

Biological pollutants, also called biocontaminants, consist of biologically originated aerosols such as

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metabolites, toxins or fragments of microorganisms that are ubiquitous in the environment (Kim et al. 2018). Indoor bioaerosol pollution can be caused by outdoor sources, building materials, furniture, occupants, pets, house plants and organic wastes (Kim et al. 2018). In addition, human activities, such as coughing, washing, talking, walking and sneezing, are also capable of generating bioaerosols (Hayleeyesus and Manaye 2014; Chen and Hildemann 2009).

The presence of undesirable aerosols, bioaerosols and chemical compounds, such as benzene (C_6H_6), formaldehyde (CH_2O) and carbon monoxide (CO), in indoor environments is an important health and social problem (WHO 2009; Kim et al. 2018; Jardim et al. 2015). Moreover, the effect of indoor pollutants on susceptible populations (patients, the elderly and pregnant women) can be very critical, due to the reduction of their immune defenses and multiple underlying chronic diseases in the case of older patients (Karottki et al. 2013; Mendes et al. 2017).

Portugal is the country with the 8th oldest population in the world and the 6th oldest in Europe, with 23% of the population over 60 years old (Almeida-Silva et al. 2014). With the increase in the elderly population, the number of nursing homes (NHs) has been growing. This elderly population spends more than 95% of their time indoors (Cheng et al. 2010; Bentayeb et al. 2013; Almeida-Silva et al. 2014), while population in general occupies about 80–90% in closed environments (Hulin et al. 2012; Nadali et al. 2020). Therefore, people should seek to be exposed to high-quality indoor air (Hayleeyesus and Manaye 2014). According to Faridi et al. (2015), due to the inability of the older people to keep healthy, the indoor air quality (IAQ) of NHs should be much better than that prevailing in other indoor environments. In this context, an assessment of the main IAQ parameters or part of them can help reducing the impact of indoor air pollution by providing information on ventilation conditions, levels of concentration of pollutants, their diversity and potential health risks to occupants of these areas (Hayleeyesus and Manaye 2014; Cheng et al. 2010).

In recent years, there has been a growing interest in indoor air quality studies in residences (Lee and Jo 2006; Stryjakowska-Sekulska et al. 2007; Balasubramanian et al. 2012; Coombs et al. 2018), hospitals (Mirzaei et al. 2014; Verde et al. 2015; Asif et al. 2018) and other places (Hayleeyesus and Manaye 2014; Zhu et al. 2003; Asadi et al. 2011; Hussina et al. 2011; Aydogdu et al. 2010; Mashat 2015; Zorpas and Skouroupatis 2016). In spite of many studies of indoor air quality in various environments, in NHs these are scarce, and the few that exist were carried out in NHs located in urban areas (Aguiar et al. 2014; Almeida-Silva et al. 2014; Faridi et al. 2015; Yilmaz et al. 2017) and other geographical world regions (Faridi et al. 2015; Yilmaz et al. 2017). These studies focus on the seasonal variations of the microbial load (Aguiar et al. 2014), identifying only fungi through phenotypic methods (Aguiar et al. 2014; Faridi et al. 2015). The characterization of some physical–chemical parameters of these environments is done separately (Almeida-Silva et al. 2014; Mendes et al. 2017). Despite these researches on air quality in NHs located in urban environments, it remains unknown whether the indoor and outdoor bacterial and fungal communities were dissimilar in summer and winter by adopting molecular approaches. In addition, as far as we know, no study has evaluated the air quality of nursing homes in rural areas. We consider that the surrounding environment is determinant for external and internal pollution. In the present study, we studied three NHs located in rural areas, under influence of Mediterranean climate, in order to quantify airborne microbial flora (bacteria and fungi) levels and its seasonal contrasts, as well as complementing the study with the evaluation of some physicochemical parameters (e.g., air temperature, relative humidity, carbon dioxide, carbon monoxide and total volatile organic compounds).

2 Material and methods

2.1 Brief description of the nursing homes

This study was carried out in three NHs (NH-A, NH-B and NH-C) located in the northeastern region of Portugal. The different buildings were built in 2013, 1996 and 2010 and improved in 2017, 2010 and 2016, respectively. The usable areas are 1700 m^2, 2700 m^2 and 1300 m^2 in NH-A, NH-B and NH-C, respectively. In relation to the layout of the compartments, in all NHs, the spaces for common use (dining room, living room, physical rehabilitation room, technical offices and laundry) are located on the ground floor, while the rooms are located on the upper floor(s). In NH-A and

NH-C, the kitchen adjoins the dining room, while in NH-B it is outside the main building. The number of residents in each NH is also distinct (44 in NH-A, 66 in NH-B and 29 in NH-C), but in all of them the area for each occupant is about 40 m^2.

All NHs are located in rural areas, close to agricultural activities, with low population density and very low road traffic. The surrounding areas are characterized by the absence of industrial activities, public car parking lots, landfills and other relevant sources of outdoor air pollution. All buildings have external double wall in plastered masonry, with insulated material between walls. The buildings have central heating systems and gas stoves in the kitchens. The ventilation of indoor spaces, in the three NHs, is natural either by air infiltration or by opening windows and doors, especially in summer, while in winter windows and doors opening is quite limited due to the low prevailing temperatures. During the visual inspection of the buildings, no evidence of moisture or mold was observed on the internal walls, ceilings, floors or furniture.

2.2 Sampling procedures

The study was performed from the beginning of 2017 till the end of 2018. Sampling periods took place in the summer of 2017 and in the winter of 2018, at each NH. Our sampling strategy was designed to best represent the environmental conditions prevailing in the different NHs during typical summer and winter days. Moreover, the measurements were carried out under normal conditions. It means that the managers of each NH were not asked to change the daily routines of their internal workers, their external service providers and their users. The samples were collected twice a day: in the morning somewhat after the beginning of NH activities and at afternoon after lunch time. In each NH, measurements/samplings were performed in four different spaces: dining room (DR), living room (LR), double bedroom (DB) and outdoor (Out) of the building. The selected indoor locations are those where residents stay longer and/or where occupancy rate can reach higher values. Outdoor sampling was performed as it is an important source of indoor air pollution and to allow comparison with the values obtained inside buildings (Jardim et al. 2015). Operational conditions were recorded, such as the entry or exit of vehicles in the home parks, burning of agricultural biomass, cleaning activities, cooking meals, number and activities performed by occupants. The cleaning of the rooms is done in the morning (9–10 am), while in the areas of common use it is done at noon (13–14 pm) and at night (20–21 pm). The elderly population spends their time talking to each other, watching television and playing games (e.g., dominoes and cards).

Indoor and outdoor measurements of the physical–chemical parameters: air temperature (T), relative humidity (RH), carbon dioxide (CO_2), carbon monoxide (CO) and total volatile organic compounds (TVOCs), were measured in each compartment (DR, LR, BD) and outside (Out) for a period about 30 min (15 min in the morning and other 15 min in the afternoon). The sampling time used by ourselves has been also adopted by several researchers in IAQ studies (Asadi et al. 2011; Haas et al. 2014). In each space the Graywolf IQ 610 probe was placed in the center of the room 1.5 m high from the ground. This probe was verified and whatever necessary it was adjusted with traceable reference gases before each measurement campaign. CO_2 was measured because it can have influence in human wealth, especially in cognitive functions at low concentrations, but principally because is a good indicator of ventilation and consequently an indicator of indoor air quality (Rodrigues and Feliciano 2019).

Total cultivable airborne bacteria and fungi were collected using a portable Surface Air System (SAS Super IAQ, International PBI, Milan), with a constant air flow rate of 100 L/min. The medium used for the collection of bacteria was Tryptic soy agar (Liofilchem, Italy) with cycloheximede, 0.5 g L^{-1}(Sigma-Aldrich, St Louis, MO, USA), while the collection of fungi was made on Rose Bengal Chloramphenicol agar (Liofilchem, Italy). Each of these measurements was made over a period of 2 min to get an air sample of 200 L. These short sampling times were chosen in order to avoid overlap of the colonies during the culture process (Haas et al. 2014) and is suggested for normal areas by the manufacturer. The sampler was placed about 1.5 m high from the floor in three central points of the compartment to assure a good representativeness of the breathing zone. For each sampling point, the air sampler was always wiped with alcohol between sample collections to avoid cross contamination. Triplicate samples for each culture medium and for each sampling period were collected for every

sampling point of each NH (DR, LR, DB and Out). Two field blanks were assessed for every NH, and no field blank samples were positive. For each season, 48 samples were taken from every NH (including 24 samples for bacteria and 24 for fungi). A total of 288 samples were collected in all NHs (144 bacterial samples and 144 fungal samples).

After sampling, the agar plates were transported to the laboratory and incubated at 37 ± 1 °C for 24–48 h for bacteria and at 25 ± 1 °C for 48–72 h for fungi. The number of colonies obtained was adjusted using the conversion table provided by the manufacturers, and the concentrations were expressed as colony-forming units per cubic meter of air ($CFU.m^{-3}$).

Single colonies were picked and cultivated on plate count agar (Liofilchem, Italy) for bacteria and potato dextrose agar (Liofilchem, Italy) plates for fungi to obtain pure isolates for subsequent identification. At first, groups of strains were formed according with their morphological similarity and then one isolated, representative of each morphotype, was selected for molecular identification.

2.3 Molecular identification of bacteria and fungi

Genomic DNA of bacterial and fungi was extracted using the REDExtract-N-Amp™ Plant PCR kit (Sigma, Poole, UK), following the manufacturer's instructions. The bacterial V1–V4 region of 16S rRNA was amplified using the primer V1f and V4r (Cai et al. 2013). Each 50 µl PCR reaction contained 2 µL of DNA extract, 1 µL dNTPs (10 mM), 5 µL buffer (10x), 1.5 µL $MgCl_2$ (25 mM), 0.5 µL of DFS-Taq DNA Polymerase (5 units/µL) and 1µL of each primer (10 µM). The samples were then placed in the MyCycler Thermal Cycler (BIO-RAD) with the following thermal profile: 1 cycle of 94° C for 2 min (initial denaturation); 30 cycles of 94 °C for 10 s (denaturation), 50 °C for 20 s (annealing) and 72 °C for 1 min (extension), followed by 1 cycle of 72 °C for 7 min (final extension).

Fungal isolates were identified by amplification of the internal transcribed spacer (ITS) region (ITS1, 5.8S, ITS2) of rRNA gene. The ITS region was amplified using ITS1/ITS4 primers sets (White et al. 1990) in a PCR protocol formerly described by Oliveira et al. (2012). The amplified products were purified and sequenced using Macrogen Inc. (Seoul, South Korea) services. Obtained sequences of DNA were analyzed using DNASTAR v.2.58 software, and species identification was performed using the NCBI (http://www.ncbi.nlm.nih.gov) and the BLAST algorithm. BLAST results were sorted according to the higher identity score and the lowest E-value. BLAST search was performed with a query coverage of ≥ 80% and ≥ 96–100% sequence similarity for assigning a species name in one of the following categories: (i) positive species identification for sequence similarity of 100%; (ii) possibly this species (suffix cf.) for sequence similarity of 99%; and (iii) certainly not this species, but taxonomic closely related (suffix aff.) for sequence similarity of 96–98%. Taxonomic classification at genus level was adopted when equal BLAST top score similarity values (ranging from 96 to 100%) were obtained for different species of the same genus or BLAST top score inferior to 96%, but with several species belonging to the same genus. Classification at the family, order and class levels was adopted when BLAST top score similarity values were < 96% for several fungi, all from the same family, order or class, respectively.

2.4 Data analysis

Statistical analyses were performed using the Software Statistical Program Social Sciences (SPSS) version 20.0 (SPSS Inc., Chicago, IL). Shapiro–Wilk test was used for normality testing. The microbial data followed a log-normal distribution; therefore, all analysis using total bacteria and fungi data were performed on the $\log_{10}$ transformed data. Data were subsequently back-transformed to assist in the reading interpretation.

Descriptive statistics were presented as arithmetic means and standard deviation. Analysis of variance (ANOVA) was used to analyze the statistical difference on physic-chemical parameters or microbial abundance among different sampling sites of each nursing home, and the averages were compared using Tukey's test ($p < 0.05$). To evaluate differences on physicochemical and microbial parameters between the two seasons (summer and winter) for the same place, t Student method was used. The indoor-to-outdoor (I/O) ratios were calculated to determine the impact of outdoor sources on indoor air concentrations.

Microbial diversity in each nursing home for the two seasons was assessed by evaluating the relative

abundance (*i.e.*, relative No. of isolates), richness (*i.e.*, No. of operational taxonomic units—OTUs) and diversity by computing Simpson's Reciprocal Index (1/D) in *Species Diversity and Richness* v. 4.0 (Seaby and Henderson 2006). The relative abundance of each OTU/genus was also determined by dividing the abundance of that OTU/genus by the total abundance of all OTUs/genera combined.

Non-metric multidimensional scaling (NMDS) was carried out to explore the similarity of microbial community composition among the indoor and outdoor of each nursing home. NMDS was performed using the Bray–Curtis similarity index (Bray and Curtis 1957). Analysis of similarity (ANOSIM) was used to evaluate the significant differences ($p < 0.05$) among the clusters obtained in NMDS ordination. This analysis compares bacterial/fungal composition between clusters and generates an R-value ranging from 0 (completely similar) to 1 (completely dissimilar) (Clarke and Gorley 2015). This analysis was conducted using the Bray–Curtis distance matrices, with 1000 permutations. When a significant difference was observed, similarity percentage analyses (SIMPER) were performed to reveal which bacterial/fungal OTUs contributed to the dissimilarity among the samples (with 0 being completely similar and 100 being completely dissimilar). All these multivariate analyses were performed by using the Community Analysis Package v. 4.0 (Henderson and Seaby 2007).

3 Main results

3.1 Physicochemical indicators

3.1.1 Air temperature and relative humidity

Figure 1 shows mean values of airT and RH registered in each monitoring site during the day (morning and afternoon) for two seasons (summer and winter). Overall, during the hot season (summer) the average indoor airT and RH were similar to the outdoor values, possibly due to the use of natural ventilation through the opening of windows and doors. On the contrary, in the cold season (winter) the average airT recorded in each compartment was significantly higher ($p < 0.05$) than outside values. As expected, indoor airT values were generally higher in the afternoon than in the morning and the indoor RH values were similar to those observed outside, except in the NH-A. Thus, the minimum and maximum airT and RH indoor varied between 17.5–22.9 °C and 37.5–58.7% in the NH-A, 19.4–26 °C and 26.8–51% in NH-B, and 18.9–24.7 °C and 29.9–56.1% in the NH-C, respectively. The lowest values of airT and the highest RH were observed in the dining room in winter season. Regarding the seasonal variation, the indoor airT values in summer in the NHs were significantly higher ($p < 0.05$) than in winter.

3.1.2 Carbon dioxide

In the hot season, the average concentrations of CO_2 inside the NHs ranged from 561 to about 850 ppm, while in cold season varied between 792 and 1881 ppm (Fig. 2). In summer, indoor/outdoor ratios (I/O) were near to 1 in all NHs (Table 1). In general, the indoor CO_2 concentrations increased in the afternoon specially in the areas where residents stay longer. The average indoor CO_2 concentrations were significantly higher ($p < 0.05$) in winter than in summer. Higher CO_2 levels were found in the living room (means of 1290 ppm in NH-A and 1701 ppm in NH-B) and dining room (means of 1881 ppm in NH-C) (Fig. 2). Also, the I/O ratio in winter was higher than 2 indicating low air renewal rates (Table 1).

3.1.3 Carbon monoxide

CO levels within NHs ranged from 0.8 to 6.4 ppm in the summer, while in the winter varied from 0.7 to 2.9. The highest mean concentrations were observed in the NH-A during summer afternoon (Fig. 2), due to external factors as demonstrated by I/O ratios, which were close to 1 in summer and higher than 2.0 in winter (Table 1).

3.1.4 Total volatile organic compounds (TVOCs)

TVOC average concentrations were significantly higher in summer than in winter ($p < 0.05$), as shown in Fig. 3. Indeed, indoor TVOC concentrations in summer were around 1677, 1379 and 1916 μg/m^3, while in winter were about 558, 1045 and 963 μg/m^3, in NH-A; NH-B and NH-C, respectively. In summer, the highest concentrations of TVOCs were observed in the DR and LR. In winter, except for NH-B, DB was the area with the highest concentrations of TVOCs.

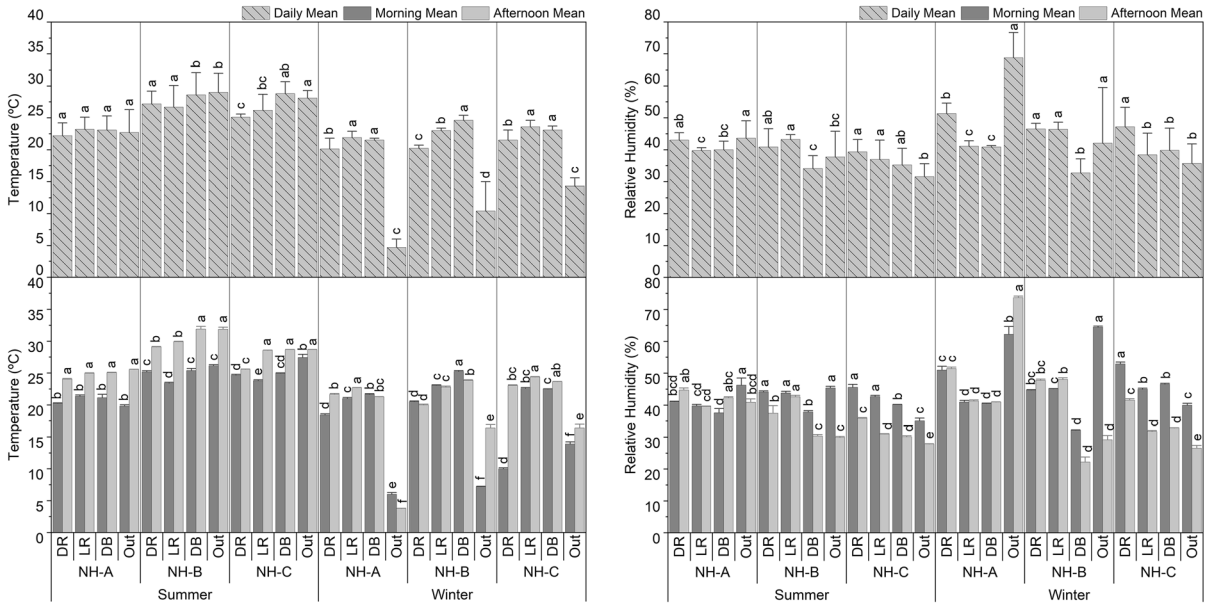

Fig. 1 Daily, morning and afternoon air temperature and relative humidity in summer and winter at the different sampling sites in each NH. Different letters by NH and sampling sites (DR, LR, BD, Out) indicate significant differences by the Tukey's test ($p < 0.05$)

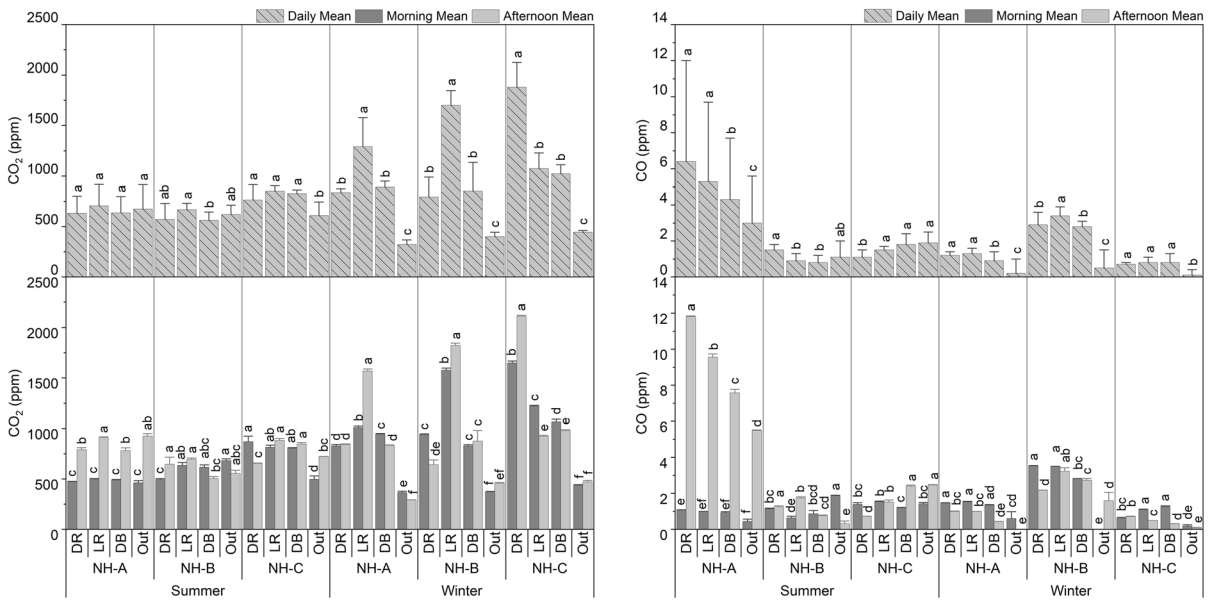

Fig. 2 Daily, morning and afternoon carbon dioxide and carbon monoxide concentrations in summer and winter at different sampling sites in each NH Different letters by NH and local sampling (DR, LR, BD, Out) indicate significant differences by the Tukey's test ($p < 0.05$)

Table 1 Indoor/outdoor ratio of chemical and biological pollutants concentrations over each nursing home

Nursing home	Summer					Winter				
	CO_2	CO	TVOC	Bacteria	Fungi	CO_2	CO	TVOC	Bacteria	Fungi
A	1.0	1.4	0.9	0.6	0.9	2.5	2.7	4.5	18.2	0.4
B	0.9	1.0	1.3	2.1	0.8	2.1	2.2	3.8	1.4	1.3
C	1.3	0.7	2.0	0.7	0.6	2.9	6.2	3.3	6.1	3.0

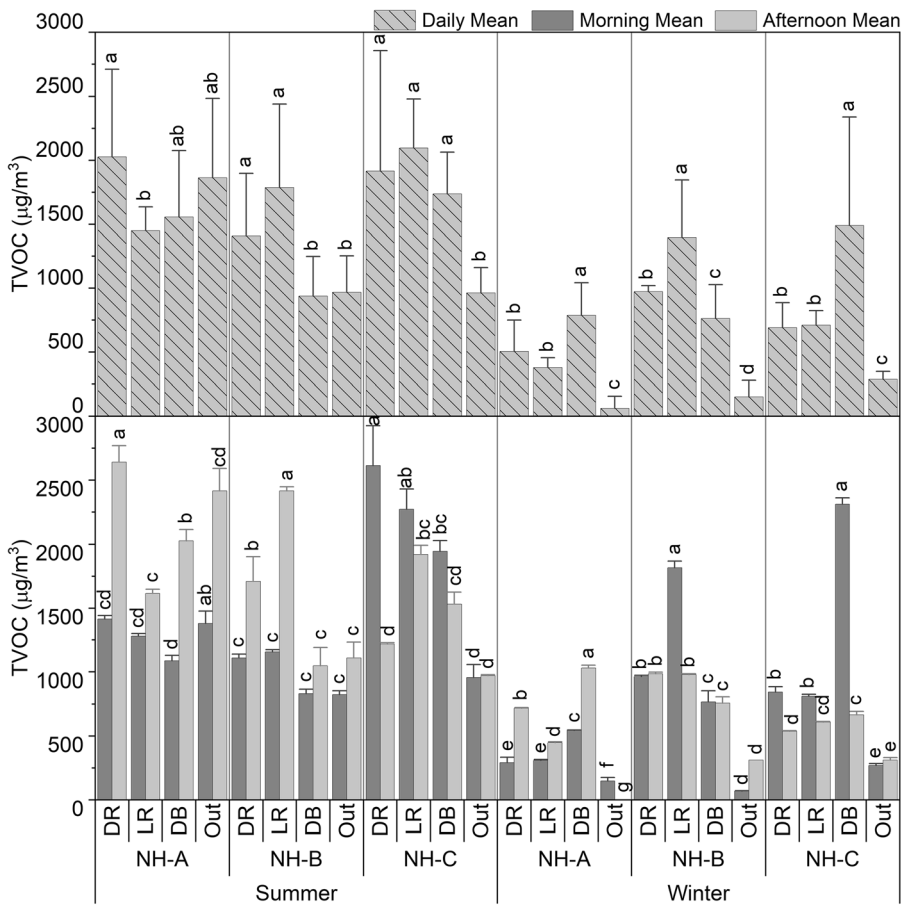

Fig. 3 Daily, morning and afternoon total volatile organic compounds concentrations in summer and winter at different sampling sites in each NH Different letters by NH and local sampling (DR, LR, BD, Out) indicate significant differences by the Tukey´s test ($p < 0.05$)

3.2 Microbial indicators

3.2.1 Bacterial and fungal abundance

Bacteria and fungi abundances, in summer and winter in different sampling sites, for nursing homes are presented in Fig. 4. In summer the average concentrations of bacteria and fungi were higher than in winter, with significant differences ($p < 0.05$) in NH-B and NH-C. Thus, the average values of bacteria and fungi in the indoor air of NHs ranged between 121–319 CFU/m^3 and 63–221 CFU/m^3 in summer, respectively, and between 2.5–179 CFU/m^3 and 21–264 CFU/m^3 in winter. Differences between morning and afternoon periods were also identified, but they were not significant for most observations

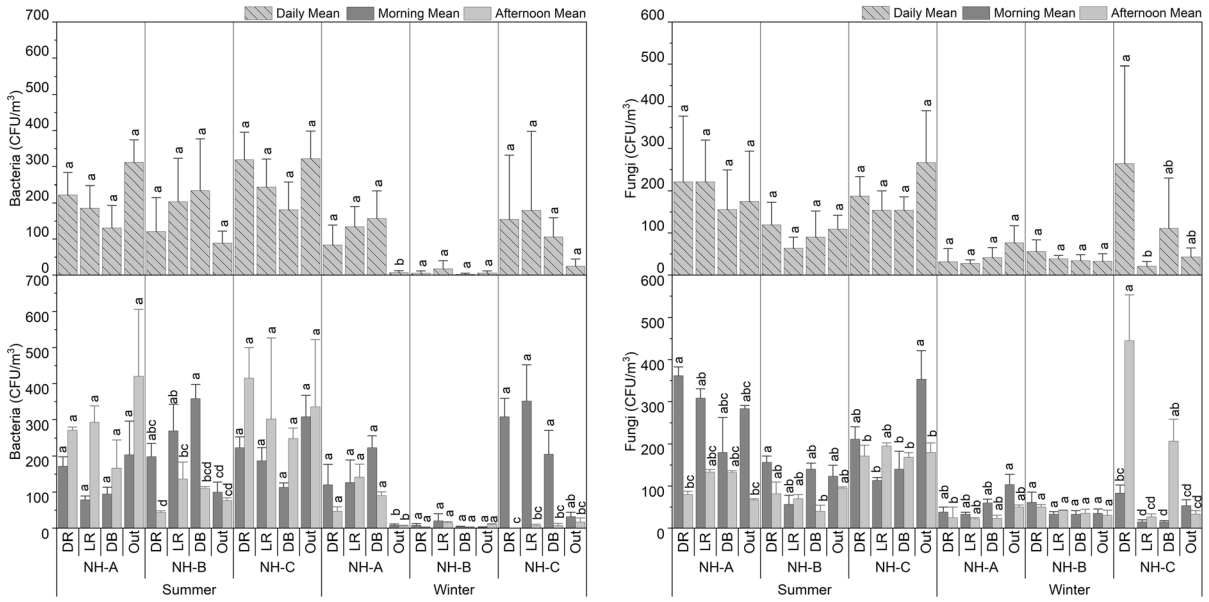

Fig. 4 Abundance of airborne bacterial and fungal in summer and winter at the different sampling sites in each NH. Different letters by NH and local sampling (DR, LR, DB, Out) indicate significant differences by the Tukey´s test ($p < 0.05$)

made in any of the NHs and compartments. Indoor/outdoor (I/O) ratios for bacteria and fungi were less than 1, in summer, except for bacteria in NH-B (I/O = 2.1), which is an indication of a greater contribution from outdoor sources (Table 1). In winter, the average concentrations of bacteria and fungi were generally higher inside than outside of buildings.

3.2.2 *Microbial diversity*

The isolation of bacteria from all NHs allowed the identification of 14 OTUs from 1003, 503 and 931 air samples collected in indoor environments and 247, 181, 301 samples collected outdoors. The most representative genus was *Staphylococcus* (39–58% in summer and 46–72% in winter), followed by *Micrococcus* (22–36% in summer and 10–26% in winter) and *Bacillus* (19–28% in summer and 17–29% in winter), while the least frequent were *Solibacillus* (3%) and *Kocuria* (1%), identified only in summer (Fig. 5).

Among the OTUs identified indoor with the highest relative abundance are *M. luteus* (24%), *S. petrasii* (31%) and *M. luteus* (37%), in the summer samples, and *S. hominis* (31%), *S. haemolyticus* (37%) and *B. simplex* (27%), in the winter samples, for the NH-A, NH-B and NH-C, respectively. In this study, the species that were less frequent in indoor air were *K. rosea* (0.1%), *E. sibiricum* (0.3%) and *S. silvestris* (3%). The diversity of bacteria in indoor and outdoor air, evaluated by the number of species (NE) and by calculating the diversity index of Shannon–Wiener (H), was higher in summer than in winter. Winter values were lower, specially outdoors, as shown in Table 2.

Concerning the fungi, ten genera were identified, namely *Alternaria, Aureobasidium, Aspergillus, Candida*, *Cladosporium, Filobasidium, Meyerozyma, Naganishia*, *Penicillium* and *Rhodotorula* (Fig. 5).

In relation to the species identified in indoor air in NH-A, NH-B and NH-C, the most abundant in summer were *Cladosporium* sp.1 (24%), *Aspergillus* sp.1 (24%) and *Penicillium* aff. *simplicissimum* (28%), while in winter were *Penicillium* aff. *glabrum* (31%), *Aspergillus* sp.1 (25%) and *Cladosporium* sp.1 (35%), respectively. Regarding the diversity of fungal species, it was observed that there is a greater diversity of species in summer than in winter, both indoors and outdoors (Table 2).

3.2.3 *Microbial community composition*

Non-metric multidimensional scaling (NMDS) allowed a clear separation of bacterial community

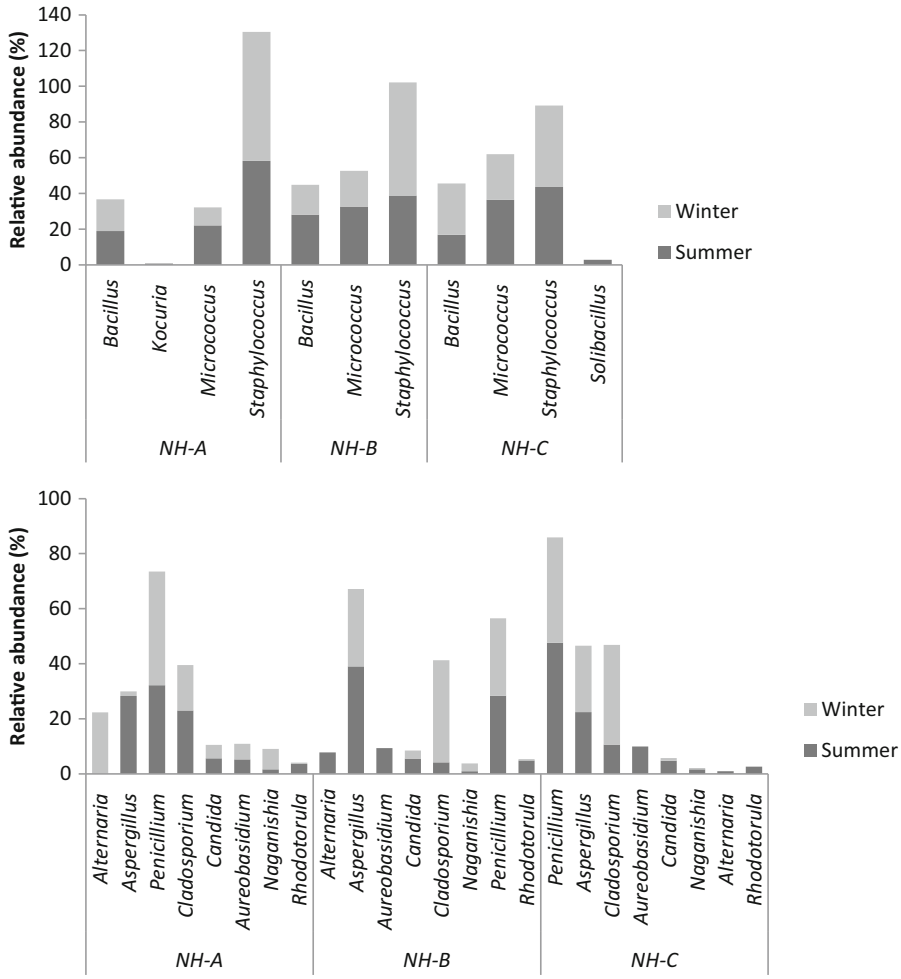

Fig. 5 Relative abundance (%) of airborne bacterial and fungal genera identified in summer and winter in the sampling sites

Table 2 Microbial diversity indices (mean ± standard deviation) over each nursing home

Nursing home		Bacteria				Fungi			
		Summer		Winter		Summer		Winter	
		H	NS	H	NS	H	NS	H	NS
A	Ind	1.6 ± 0.1	9	1.2 ± 0.3	5	1.9 ± 0.5	10	1.1 ± 0.2	4
	Out	1.5 ± 0.0	9	0.3 ± 0.4	3	1.7 ± 0.2	8	1.7 ± 0.2	4
B	Ind	1.4 ± 0.1	9	0.2 ± 0.4	4	1.5 ± 0.5	7	1.4 ± 0.3	6
	Out	1.2 ± 0.1	6	0.2 ± 0.3	4	1.7 ± 0.2	7	0.9 ± 0.5	4
C	Ind	3.2 ± 0.1	6	0.9 ± 0.7	7	1.8 ± 0.2	9	1.2 ± 0.5	6
	Out	1.3 ± 1.1	5	0.7 ± 0.6	5	1.7 ± 0.3	9	1.3 ± 0.4	5

H Shannon–Wiener; *NS* number of OTUs; *Ind* indoor; *Out* outdoor

into summer and winter groups (Fig. 6). This differentiation was also corroborated by ANOSIM, which showed a significant difference in bacterial compositions between summer and winter ($R = 0.83$, $p = 0.001$). This difference was much more evident indoors ($R = 0.90$, $p = 0.001$) than outdoors ($R = 0.66$, $p = 0.01$). According to the SIMPER analysis, *S. petrasii* in NH-A (17%) and NH-B (27%) and *S. haemolyticus* (26%) in the NH-C were

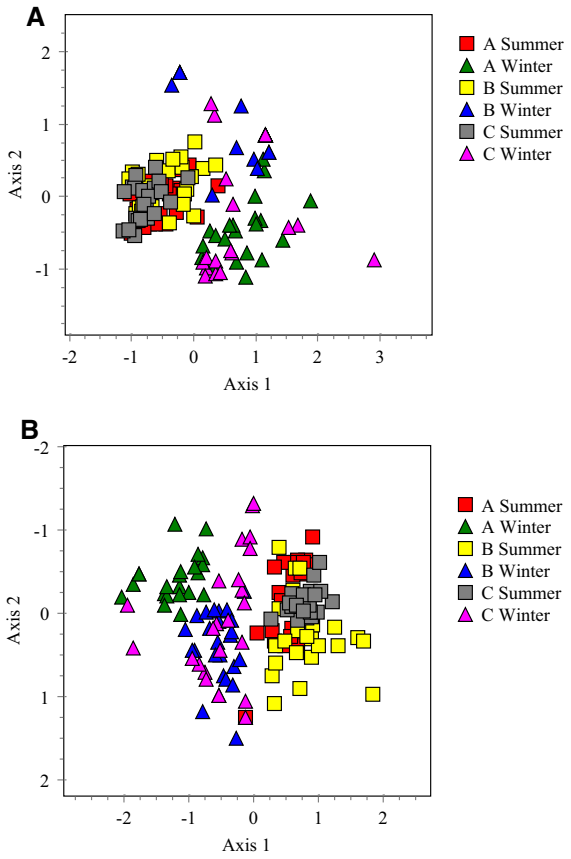

Fig. 6 Non-metric multidimensional scale (NMDS) plot corresponding to the clustering of bacterial (A) and fungal (B) communities grouped by summer (squares) and winter (triangles) in each nursing home (A, B, and C)

the species that contributed the most to these dissimilarities.

Non-metric multidimensional scaling (NMDS) and ANOSIM analysis showed that the fungal community identified in summer was significantly different from that identified in winter ($R = 0.80, p = 0.001$) (Fig. 5). This difference was more noticeable in the indoor ($R = 0.82$, $p = 0.001$) than in the outdoor ($R = 0.72$, $p = 0.001$). According to the SIMPER analysis, *Cladosporium* sp. 1 (15%), *Aspergillus* sp. 1 (14%) and *Penicillium* aff. *simplicissimum* (25%) were the species that contributed most to the dissimilarity of the fungal community between summer and winter in NH-A, NH-B and NH-C, respectively.

4 Discussion

The elderly people spend 95% of their time indoors divided between bedrooms and living rooms (Almeida-Silva et al., 2014). Thus, IAQ is important for this sensitive group of people. In our study, indoor airT and RH values found in summer and winter are in accordance with those recommended by the American Society of Heating, Refrigeration and Air Conditioning Engineer, which recommends temperatures around 23–26 °C and relative humidity between 30–60% (Asadi et al. 2011), and by the Engineering Institute of Thailand (EIT), which recommends temperatures ranging from 24 to 27 °C and relative humidity below 60% (Tungjai and Kubaha 2017). According to Ormandy and Ezratty (2012), susceptible groups need to be at a minimal temperature of 20 °C to avoid cardiovascular issues. Mourtzoukou and Falagas (2007) also reported that cold air inhalation or cooling of a part of the body surface is associated with an increased risk of respiratory infections. However, in the all NHs studied during the cold season, the average values of airT were quite steady and above 20 °C.

In the present work, the lowest airT values and the highest RH recorded in the dining room in winter may be related to the characteristics of DR, kitchen (water vapor source) contiguous to the dining room, especially in NH-A and NH-C, and the low occupancy of this space (only during the meal period).

In relation to CO_2, higher levels were found in the living room (NH-A and NH-B) and dining room (NH-C) slightly above the protection limit (1250 ppm) established by the Portuguese legislation (Ordinance n.° 353-A/ 2013). Also, the I/O ratio in winter was higher than 2 indicating low air renewal rates. This finding can be attributed to the fact that during the cold season the windows are closed along most of the day and residents spend more time inside the buildings (living and dining rooms) and therefore CO_2 concentrations increase. It should be noted that occupants' breathing is the most relevant source of carbon dioxide in these spaces. According to Canha et al. (2017), low ventilation rates promote the indoor accumulation of pollutants, maximizing the exposure levels of the occupants.

The highest indoor CO concentration values were observed in summer and its origin was associated with external sources, as shown by the I/O ratios. This is

justified by the frequent opening of windows and doors that serve as an entrance zone of air pollutants from neighborhood sources (automobiles and agricultural biomass burnings). However, the short mean values achieved have not exceeded the specific conditions used for checking the conformity of CO in situations of short-term exceedance (Ormandy and Ezratty 2012). Furthermore, CO values were systematically well below the limit of 9 ppm averaged over an 8 h exposure period established by Portuguese Ordinance n.° 353-A/2013. On contrary, in winter the dominant sources of CO were internal (I/O > 1). The most important source of exposure to CO in indoor air, in developed countries, are emissions from faulty, incorrectly installed, poorly maintained or poorly ventilated cooking or heating appliances that burn fossil fuels (WHO 2010). In addition, incense burning in indoor spaces can be also a source of exposure to carbon monoxide (WHO 2010). Thus, the proximity of the kitchens to the DR in NH-A and NH-C and the use of incense in order to eliminate odors, associated with poor ventilation of the spaces, explain indoor CO concentrations in those NHs.

In all NHs, TVOC concentrations in indoor air compartments were higher than the limit values established by Portuguese legislation, 600 μg/m^3 with 100% tolerance margin (Ordinance n.° 353-A/ 2013). In the cool season the I/O ratio was always higher than 3 in all NHs (Table 1). Our findings corroborate with those observed by Yang et al. (2009) in school buildings in Korea and by Langer et al. (2015) in new houses in Sweden. Regular use of air fresheners and cleaning products may be responsible for high concentrations of TVOCs. This cause–effect relationship was also referred by Asadi et al. (2011) and Tungjai and Kubaha (2017). The recent rehabilitation of buildings may also be at the origin of this problem, since TVOCs can be emitted from the paint and glues used (Jardim et al. 2015). Thus, as stated in Portuguese legislation (Ordinance n.° 353-A/ 2013), a TVOCs speciation analysis must be performed for the identification of the main volatile organic compounds (benzene, trichlorethylene, toluene, styrene and tetrachlorethylene), as well as identify its main sources and its potential risks to human health.

TVOC concentrations did not show a characteristic morning/afternoon contrast. This finding can be partly explained by some irregularity in the cleaning pattern and in the behavior of the residents throughout the daytime hours, making difficult repeat all sampling/monitoring action under de same environmental conditions. Nevertheless, morning/afternoon contrasts were identified for CO_2 concentrations, existing a slight tendency for higher afternoon values, due to human occupation and activities. However, in general, the differences were not statistically significant.

Concerning the bacteria and fungi concentrations, the present study shows a similarity in the concentrations of bacteria and fungi inside and outside of the NHs in summer (Fig. 4). Regarding the daily variation, there are differences in bacterial and fungal concentrations in the morning and afternoon as found in other studies (e.g., Hameed et al., 2009), but the differences were not statistically significant for most cases and no specific variation pattern was found between morning and afternoon bacterial and fungal levels. I/O less than 1 in summer was also reported by Faridi et al. (2015) in retirement home (in Tehran city) located in residential area (1.73 and 0.85 for bacteria and fungi, respectively). In winter, residents tend to stay inside living rooms, in larger number and for longer periods (Almeida-Silva et al. 2014), under lower fresh air renewal rates, which contributes for increasing indoor biocontaminant concentrations (I/O > 1). Similar results were obtained by Faridi et al. (2015) in the retirement home (I/O = 1.33 for bacteria and I/O = 1.77 for the fungal spores). Nevertheless, overall concentrations of bacteria and fungi did not exceed the reference values established by Portuguese legislation (Ordinance n.° 353-A/ 2013), which recommends indoor fungi concentrations less than outdoor concentrations; and for bacteria, the indoor concentration should not exceed the outdoor concentration by 350 CFU/m^3. Also, if we consider the classification for bacteria and fungi presented by Neto and Siqueira (2000) (i.e., I/O ≤ 1.5 good, I/O = 1.5 up to 2.0 regular and I/O > 2.0, poor indoor ambient conditions), we can assume that indoor air quality for bacteria and fungi was good in summer in NH-A and NH-C, and regular in NH-B. In winter, only NH-B would rank well, while NH-A and NH-C were poor. The existing seasonality showed by our results is corroborated by other researchers (Zhai et al., 2018) who reported that microbial air concentrations in the same season do not show significant changes, due to seasonal microbial stability, while in different seasons and sampling locations differences are noticeable. Despite the number of samples seemed to be

insufficient to obtain a good temporal representativeness, the strategy adopted in this work proved suitable to observe a similar pattern of seasonal variation between nursing homes, which is in accordance with previous studies (Yilmaz et al. 2017).

In our study, the most representative genera in the air were Gram-positive, namely *Staphylococcus*, *Micrococcus* and *Bacillus*. These bacteria are usually the most prevalent indoors and outdoors, in all season, in hospitals (Mirzaei et al. 2014), public buildings (Awad et al. 2018), school dormitories and retirement homes (Faridi et al. 2015). The abundance of these bacteria both in indoors and outdoors air is related to their lifestyle (saprophism and commensalism) and ability to survive even in unfavorable environmental conditions, such as dryness, intensive solar radiation and chemical pollutants (Dietze et al. 2001). *Staphylococcus* and *Micrococcus* are commonly found on human skin (Awad et al. 2018; Madsen et al. 2018) and are associated with opportunistic infections (Takeuchi et al. 2005). *Bacillus* as endospore-forming bacteria is widely distributed in soil habitats. This explains its aerial distribution and subsequent occurrence in many indoor environments (Gołofit-Szymczak and Górny 2010). In addition, the abundance of these genera is also related to the conditions presented by most buildings, specifically air temperatures ranging from 20 to 30 °C and moderate RH (40–60%) (Awad et al. 2018). In general, these conditions were verified in all NHs. Other species, such as *K. rosea*, *E. sibiricum* (0.3%) and *S. silvestris* (3%), were less frequent in this study and their occurrence in indoor environments is not widely reported and, when referred to, its prevalence is lower in relation to the other identified species. Our study also showed a greater diversity of the bacterial community in the indoor and outdoor air of NHs in summer than in the winter.

Among the most common fungal species were those from genera *Aspergillus*, *Cladosporium* and *Penicillium* (Fig. 5), which is consistent with the findings of the previous studies in NHs located in urban areas (Aguiar et al. 2014; Faridi et al. 2015; Yilmaz et al. 2017). For most indoor fungi, the optimum temperature and relative humidity for growth are 10–35 °C and < 90% (WHO 2009), respectively, values that are observed inside NHs. In addition, the high prevalence of airborne mold was also related to its ability to survive in extreme environmental conditions (temperature and relative humidity) (Sharma 2005) and also their xerophilic character, which makes them able to grow on soil, dust, surface of building materials, seeds and many foods (Jeddi et al. 2014). The airborne fungi identified in the present work are commonly associated with allergic diseases (Knudsen et al. 2017; Reddy and Srinivas 2017). Regarding the diversity of fungal species, it was found that there is a greater diversity of species in summer than in winter, similarly to what was observed with bacteria.

5 Conclusions and recommendations

Globally, the measured indoor contaminants levels suggest that the three spaces studied have a satisfactory air quality, although it seems evident that indoor air quality is poorer during winter. Moreover, as these spaces are occupied by the elderly, it cannot be definitively concluded that such levels do not endanger the health of the occupants.

In relation to airborne bacteria and fungi, the most abundant bacterial genera were *Bacillus*, *Micrococcus* and *Staphylococcus*, while the dominant fungal genera were *Aspergillus*, *Cladosporium* and *Penicillium*. Some of the bacterial and fungal species identified in this study may cause opportunistic infections. Examples of these species are *S. petrasii, S. epidermidis* and *M. luteus* and some species of *Aspergillus* genus. However, the concentrations obtained in this study are within the legal limits.

Outdoor conditions ambient appeared to not affect indoor concentrations since the overall I/O ratios were equal to or greater than 1.0. Therefore, further studies need to be carried out to ascertain the possible internal sources of pollution.

In view of the results obtained in this study, an effective and accessible proposal for the three NHs studied is to promote higher rates of natural ventilation especially in winter, in order to increase fresh air renewal and consequently assure a higher dilution rate of pollutants. In addition, the use of natural ventilation should take into account the influence of external air pollution sources.

Particular attention should be paid to kitchens and bathrooms, as they may be fungal growth sites due to the high moisture content. In the case of kitchens, a measure that could be taken would be to isolate them

from the rest of the building, similar to NH-B, or prevent direct communication with the dining room.

In a future research, it is important to include other pollutants (e.g., PM_{10}, $PM_{2.5}$) and deepen the analysis of TVOCs, by identifying the most prevailing in those type of buildings and to evaluate potential consequences on the health of the occupants.

Acknowledgements The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support by national funds FCT/MCTES to CIMO [UIDB/00690/2020]. The authors would also like to acknowledge Leonardo Furst for the support provided in the elaboration of some figures, as well as all the members of each nursing home team for his unconditional support throughout the study.

References

Aguiar, L., Mendes, A., Pereira, C., Neves, M. P., & Teixeira, P. J. (2014). Contaminação microbiológica do ar em lares da 3 idade na cidade do Porto. Projeto GERIA. *Observações - Boletim Epidemiológico, artigos breves, 10,* 33–36.

Almeida-Silva, M., Wolterbeek, H. T., & Almeida, S. M. (2014). Elderly exposure to indoor air pollutants. *Atmospheric Environment, 85,* 54–63. https://doi.org/10.1016/j.atmosenv.2013.11.061.

Asadi, E., Costa, J. J., & Silva, G. M. (2011). Indoor air quality audit implementation in a hotel building in Portugal. *Building and Environment, 46,* 1611–1623. https://doi.org/10.1016/j.buildenv.2011.01.027.

Asif, A., Zeeshan, M., Hashmi, I., Zahid, U., & Bhatti, F. M. (2018). Microbial quality assessment of indoor air in a large hospital building during winter and spring seasons. *Building and Environment, 135,* 68–73. https://doi.org/10.1016/j.buildenv.2018.03.010.

Awad, H. A., Saeed, Y., Hassan, Y., Fawzy, Y., & Osman, M. (2018). Air microbial quality in certain public buildings, Egypt: A comparative study. *Atmospheric Pollution Research, 9,* 617–626. https://doi.org/10.1016/j.apr.2017.12.014.

Aydogdu, H., Asan, A., & Otkun, T. M. (2010). Indoor and outdoor airborne bacteria in child day-care centers in Edirne City (Turkey), seasonal distribution and influence of meteorological factors. *Environmental Monitoring and Assessment, 164,* 53–66. https://doi.org/10.1007/s10661-009-0874-0.

Balasubramanian, R., Nainar, P., & Rajasekar, A. (2012). Airborne bacteria, fungi, and endotoxin levels in residential microenvironments: A case study. *Aerobiologia, 28,* 375–390. https://doi.org/10.1007/s10453-011-9242-y.

Bentayeb, M., Viegi, G., Simoni, M., Norback, D., Baldacci, S., Maio, S., et al. (2013). Indoor air pollution and respiratory health in the elderly. *Journal of Environmental Science and Health, Part A, 48,* 37–41.

Bray, J. R., & Curtis, J. T. (1957). An ordination of the upland forest communities of southern Wisconsin. *Ecological Monographs*. https://doi.org/10.2307/1942268.

Cai, L., Ye, L., Tong, Y. H. A., Lok, S., & Zhang, T. (2013). Biased diversity metrics revealed by bacterial 16S pyrotags derived from different primer sets. *PLoS ONE, 8*(1), e53649.

Canha, N., Lage, J., Candeias, S., Alves, C., & Almeida, M. S. (2017). Indoor air quality during sleep under different ventilation patterns. *Atmospheric Pollution Research, 8,* 1132–1142.

Chen, Q., & Hildemann, L. M. (2009). The effects of human activities on exposure to particulate matter and bioaerosols in residential homes. *Environmental Science and Technology, 43*(13), 4641–4646. https://doi.org/10.1021/es802296j.

Cheng, J. T., Chang, Y. C., Tsou, N. P., Wu, J. M., & Feng, S. Y. (2010). The determinants of mass concentration of indoor particulate matter in a nursing home. *Applied Mechanics and Materials, 44,* 3026–3030. https://doi.org/10.4028/www.scientific.net/AMM.44-47.3026.

Clarke, K. R., & Gorley, R. N. (2015). *PRIMER v7: User Manual/Tutorial*. Plymouth: PRIMER-E Ltd.

Coombs, K., Taft, D., Ward, V. D., Green, B. J., Chew, G. L., Shamsaei, B., et al. (2018). Variability of indoor fungal microbiome of green and non-green low-income homes in Cincinnati, Ohio. *Science of the Total Environment, 610,* 212–218. https://doi.org/10.1016/j.scitotenv.2017.07.274.

Dietze, B., Rath, A., Wendt, C., & Martiny, H. (2001). Survival of MRSA on sterile goods packaging. *Journal of Hospital Infection, 49,* 255–261. https://doi.org/10.1053/jhin.2001.1094.

Faridi, S., Hassanvand, S. M., Naddafi, K., Yunesian, M., Nabizadeh, R., Sowlat, H. M., et al. (2015). Indoor/outdoor relationships of bioaerosol concentrations in a retirement home and a school dormitory. *Environmental Science and Pollution Research, 22,* 8190–8200. https://doi.org/10.1007/s11356-014-3944-y.

Gołofit-Szymczak, M., & Górny, R. L. (2010). Bacterial and fungal aerosols in air-conditioned office buildings in Warsaw, Poland—the winter season. *International Journal of Occupational Safety and Ergonomics, 16*(4), 465–476.

Haas, D., Habib, J., Luxner, J., Galler, H., Zarfel, G., Schlacher, R., et al. (2014). Comparison of background levels of culturable fungal spore concentrations in indoor and outdoor air in southeastern Austria. *Atmospheric Environment, 98,* 640–647.

Hameed, A. A. A., Khoder, M. I., Yuosra, S., Osman, A. M., & Ghanem, S. (2009). Diurnal distribution of airborne bacteria and fungi in the atmosphere of Helwan area, Egypt. *Science of the Total Environment, 407,* 6217–6222. https://doi.org/10.1016/j.scitotenv.2009.08.028.

Hayleeyesus, S. F., & Manaye, A. M. (2014). Microbiological quality of indoor air in university libraries. *Asian Pacific Journal of Tropical Biomedicin, 4*(Suppl 1), S312–S317. https://doi.org/10.12980/APJTB.4.2014C807.

Henderson, P. A., & Seaby, R. M. H. (2007). *Community analysis package 4.0*. Lymington: Pisces Conservation Ltd.

Hulin, M., Simoni, M., Viegi, G., & Annesi-Maesano, I. (2012). Respiratory health and indoor air pollutants based on

quantitative exposure assessments. *European Respiratory Journal, 40*(4), 1033–1045. https://doi.org/10.1183/09031936.00159011.

Hussina, N. H. M., Sann, L. M., Shamsudin, M. N., & Hashim, Z. (2011). Characterization of bacteria and fungi bioaerosol in the indoor air of selected primary schools in Malaysia. *Indoor and Built Environment*. https://doi.org/10.1177/1420326X11414318.

Jardim, D, Diegues, P., Santiago, A., Matias, P., Reis, V., Matos, J., et al. (2015). Metodologia de avaliação da qualidade do ar no interior de edifícios de comércio e serviços no âmbito da Portaria 353-A/2013, de 4 de dezembro. Agência Portuguesa do Ambiente em parceria com a Direção-Geral da Saúde. Retrieved March 10, 2020, from http://apambiente.pt/_zdata/DAR/Ar%20Interior/Metodologia_Avaliacao_Qualidade_Ar_Interior_1.0.pdf.

Jeddi, M. Z., Yunesian, M., Gorji, M. E., Noori, N., Pourmand, M. R., & Khaniki, G. R. (2014). Microbial evaluation of fresh, minimally-processed vegetables and bagged sprouts from chain supermarkets. *Journal of Health, Population and Nutrition, 32,* 391–399.

Karottki, D. G., Spilak, M., Frederiksen, M., Gunnarsen, L., Brauner, E. V., Kolarik, B., et al. (2013). An indoor air filtration study in homes of elderly: Cardiovascular and respiratory effects of exposure to particulate matter. *Environmental Health, 12,* 1–10.

Kim, K. H., Kabir, E., & Jahan, S. A. (2018). Airborne bioaerosols and their impact on human health. *Journal of Environmental Sciences, 67,* 23–35. https://doi.org/10.1016/j.jes.2017.08.027.

Knudsen, M. S., Gunnarsen, L., & Madsen, M. A. (2017). Airborne fungal species associated with mouldy and non-mouldy buildings e effects of air change rates, humidity, and air velocity. *Building and Environment, 122,* 161–170. https://doi.org/10.1016/j.buildenv.2017.06.017.

Langer, S., Beko, G., Bloom, E., Widheden, A., & Ekberg, L. (2015). Indoor air quality in passive and conventional new houses in Sweden. *Building and Environment, 93,* 92–100. https://doi.org/10.1016/j.buildenv.2015.02.004.

Lee, J.-H., & Jo, W.-K. (2006). Characteristics of indoor and outdoor bioaerosols at Korean high-rise. apartment buildings. *Environmental Research, 101,* 11–17. https://doi.org/10.1016/j.envres.2005.08.009.

Madsen, M. A., Jenabian, M. S., Islam, Z. M., Frankel, M., Spilak, M., & Frederiksen, W. M. (2018). Concentrations of *Staphylococcus* species in indoor air as associated with other bacteria, season, relative humidity, air change rate, and *S. aureus* positive occupants. *Environmental Research, 160,* 282–291.

Mashat, B. (2015). Indoor and outdoor microbial aerosols at the holy mosque: A case study. *Atmospheric Pollution Research, 6,* 990–996. https://doi.org/10.1016/j.apr.2015.05.004.

Mendes, A., Papoila, A. L., Carreiro-Martins, P., Aguiar, L., Bonassi, S., Caires, I., et al. (2017). The influence of thermal comfort on the quality of life of nursing home residents. *Journal of Toxicology and Environmental Health, Part A, 80*(13–15), 729–739. https://doi.org/10.1080/15287394.2017.1286929.

Mirzaei, R., Shahriary, E., Qureshi, I. M., Rakhshkhorshid, A., Khammary, A., & Mohammadi, M. (2014). Quantitative and qualitative evaluation of bio-aerosols in surgery rooms and emergency department of an educational hospital. *Jundishapur Journal of Microbiology, 7,* 1–5.

Mourtzoukou, E. G., & Falagas, M. E. (2007). Exposure to cold and respiratory tract infections. *International Journal of Tuberculosis and Lung Disease, 11,* 938–943.

Nadali, A., Arfaeinia, H., Asadgol, Z., & Fahiminia, M. (2020). Indoor and outdoor concentration of PM_{10}, $PM_{2.5}$ and PM_1 in residential building and evaluation of negative air ions (NAIs) in indoor PM removal. *Environmental Pollutants and Bioavailability, 32*(1), 47–55. https://doi.org/10.1080/26395940.2020.1728198.

Neto, F. R. A., & Siqueira, L. F. G. (2000). Guidelines for indoor air quality in offices in Brazil. *Proceedings of Healthy Buildings, 4,* 549–554.

Oliveira, L., Pereira, J. A., Lino-Neto, T., Bento, A., & Baptista, P. (2012). Fungal diversity associated to the olive moth, *Prays oleae* Bernard: a survey for potential entomopathogenic fungi. *Microbial Ecology, 63,* 964–974.

Ordinance n.° 353-A/2013 of 4th December (2013). Diário da República, n.° 235/2013, 1st serie. Ministry of environment, territory planning, health and solidarity, employment and social security, Lisbon, Portugal. https://data.dre.pt/eli/port/353-a/2013/p/cons/20140131/pt/html.

Ormandy, D., & Ezratty, V. (2012). Health and thermal comfort: From WHO guidance to housing strategies. *Energy Policy, 49,* 116–121. https://doi.org/10.1016/j.enpol.2011.09.003.

Reddy, K. M., & Srinivas, T. (2017). Mold allergens in indoor play school environment. *Energy Procedia, 109,* 27–33.

Rodrigues, F., & Feliciano, M. (2019). Improving indoor air quality of naturally ventilated classrooms in the northeast of Portugal. *Environmental Engineering and Management Journal, 18,* 1423–1437.

Sattar, S. A., & Bact, D. (2016). Indoor air as a vehicle for human pathogens: Introduction, objectives, and expectation of outcome. *American Journal of Infection Control, 44*(9 Suppl), S95–S101. https://doi.org/10.1016/j.ajic.2016.06.010.

Seaby, R. M., & Henderson, P. A. (2006). *Species Diversity and richness. Version 4*. Lymington: Pisces Conservation Ltd.

Sharma, P. D. (2005). *Fungi and allied organisms*. Oxford: Alpha Science International Ltd.

Stryjakowska-Sekulska, M., Piotraszewska-Pająk, A., Szyszka, A., Nowicki, M., & Filipiak, M. (2007). Microbiological quality of indoor air in university rooms. *Polish Journal of Environmental Studies, 16,* 623–632.

Takeuchi, F., Watanabe, S., Baba, T., Yuzawa, H., Ito, T., Morimoto, Y., et al. (2005). Whole-genome sequencing of *Staphylococcus haemolyticus* uncovers the extreme plasticity of its genome and the evolution of human-colonizing staphylococcal species. *Journal of Bacteriology, 187*(21), 7292–7308.

Tungjai, A., & Kubaha, K. (2017). Indoor air quality evaluation of isolation room assessing the feasibility of using the heat demand-outdoor. *Energy procedia, 138,* 858–863.

Verde, C. S., Almeida, M. S., Matos, J., Guerreiro, D., Meneses, M., Faria, T., et al. (2015). Microbiological assessment of indoor air quality at different hospital sites. *Research in Microbiology, 166,* 557–563. https://doi.org/10.1016/j.resmic.2015.03.004.

WHO—World Health Organization. (2009). WHO guidelines for indoor air quality: Dampness and mould. World Health Organization Regional Office for Europe, Copenhagen. Retrieved January, 2019, from https://www.euro.who.int/__data/assets/pdf_file/0017/43325/E92645.pdf.

WHO—World Health Organization. (2010). Guidelines for indoor air quality: Selected pollutants. World Health Organization Regional Office for Europe, Copenhagen. Retrieved January, 2019, from https://www.euro.who.int/__data/assets/pdf_file/0009/128169/e94535.pdf.

Yang, W., Sohn, J., Kim, J., Son, B., & Park, J. (2009). Indoor air quality investigation according to age of the school buildings in Korea. *Journal of Environmental Management, 90,* 348–354.

Yilmaz, O., Asan, A., Aydogdu, H., & Sem, B. (2017). Airborne fungal diversity inside a nursing home in Edirne, Turkey. *Fresenius Environmental Bulletin, 26*(12), 7025–7033.

Zhai, Y., Li, X., Wang, T., Wang, B., Li, C., & Zeng, G. (2018). A review on airborne microorganisms in particulate matters: Composition, characteristics and influence factors. *Environment International, 113,* 74–90. https://doi.org/10.1016/j.envint.2018.01.007.

Zhu, H., Phelan, P., Duan, T., Raupp, G., & Fernando, S. J. H. (2003). Characterizations and relationships between outdoor and indoor bioaerosols in an office building. *China particuology, 3,* 119–123. https://doi.org/10.1016/S1672-2515(07)60122-5.

Zorpas, A., & Skouroupatis, A. (2016). Indoor air quality evaluation of two museums in a subtropical climate conditions. *Sustainable Cities and Society, 20,* 52–60. https://doi.org/10.1016/j.scs.2015.10.002.