Resumo

Humidade e disponibilidade de água nos materiais de construção em edifícios podem contribuir para o crescimento microbiano. A presença de partículas em ambiente interior pode estar relacionada com efeitos sobre a saúde. Foi realizada uma avaliação da exposição a bioaerosossís e a partículas. Quinze amostras de ar interior e uma do exterior, para servir de referência, foram realizadas. Amostras de superfícies do pavimento e das grelhas de ventilação foram também efectuadas. A identificação de espécies fúngicas específicas sugere a implementação de medidas correctivas e as partículas são provavelmente introduzidas em ambiente interior através do sistema de ventilação. Os resultados obtidos confirmam a exposição ocupacional a bioaerosossís e partículas e a necessidade de intervenção no sistema de ventilação.

Palavras-chave: Bioaerosóis; Fungos; Bactérias; Partículas; Exposição ocupacional.

Abstract

Excessive humidity and/or high water content in building materials can contribute to microbial growth. The presence of particles indoors can be related to several adverse health outcomes. An assessment was performed comprising bioaerosols sampling and measurements of particles. Fifteen indoor samples were done and one outdoor sample, to be used as a reference. Surface samples from floor and ventilation grids were performed in the same indoor spaces. The identification of specific fungal species requires implementation of corrective measures and particles are probably introduced in indoor environment by the ventilation system. Results obtained confirm occupational exposure to bioaerosols and particles and the need for intervention in the ventilation system.

Keywords: Bioaerosols; Fungi; Bacteria; Particles; Occupational exposure
1. Theory

Occupational exposure to bioaerosols has become a subject of concern over recent years due to the related 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). Indoor sources of airborne bacteria in buildings include the presence of humans, pets, soils, and plants (Bowers et al., 2012; Lignell, 2008; Womack et al., 2010). Fungal spores in outdoor air are a major source for indoor fungi during spring and summer for naturally ventilated buildings (WHO, 2009). However, fungal indoor reservoirs should also be considered (WHO, 2009; Crawford et al., 2015). Growth conditions like excessive humidity and/or high water content in building materials can be the promoting factor for microbial growth. This is often caused by lack of thermal insulation, as well as the incorrect behaviour of workers/occupants (WHO, 2009).

The presence of particles indoors can be related to several adverse health outcomes, particularly in the cardiovascular and respiratory systems (Kreiling et al., 2006; Pope and Dockery, 2006; Schmid et al., 2009; Cesaroni et al., 2013). The potential of particles to generate adverse local (respiratory system) and systemic health effects is related to their capacity to reach the lungs. In theory, particles can carry several toxic compounds and biologic agents with them and with this transporting the chemical and biological agents to the target biological systems (Buonanno et al., 2014). Currently, it still not known which particle size, morphology or chemical and biological composition is most intensely related to the adverse health outcomes and further research in needed to clarify this aspect (Buonanno et al., 2014).

In terms of particle size, attention has moved from mass (PM10 or PM2.5 - mass concentration of particles smaller than 10 μm and 2.5 μm in aerodynamic diameter, respectively) to surface area and particle number concentrations (Giechaskiel et al., 2009; Franck et al., 2011; Cauda et al., 2012; Buonanno et al., 2014). Additionally, previous research showed that particles indoor concentrations were significantly related to the corresponding outdoor concentrations, but the relationship varied with the aerodynamic particle diameter and composition (Lin and Tai, 1998).

The aim of this study was to assess occupational exposure to bioaerosols and particles in one office building where all workers presented several health complaints, mainly in respiratory system.

2. Methodology

An assessment was performed comprising bioaerosols sampling and measurements of particles. This campaign was done during 20th of April of 2017 and started after 10 a.m. with the building office working at normal conditions and with the usual number of occupants. The building has a mechanical ventilation and acclimatization system and each office has also a window that was closed during the assessment.

Measurements and sampling were done in each workplace that corresponded to different offices with one worker each. The tasks develop normally in each office are essentially administrative that imply the use of a computer and
phones. In one of the offices (5G1) was also present a printer that was used in a regular basis.

2.1. Bioaerosols sampling

Air samples consisted mainly of fifteen indoor samples (five for fungi and ten for bacteria – five for each different media) and one outdoor sample, to be used as a reference. All indoor samples were collected from offices occupied per one or two workers. Air samples of 250 L were collected through an impaction method with a flow rate of 140 L/min (Millipore air Tester, Millipore, Billerica, MA, USA) onto each media plate according to manufacturer’s instructions. Two different culture media were used in order to enhance the selectivity for bacterial and fungal populations growth: malt extract agar (MEA) supplemented with chloramphenicol (0.05%) was used for fungi and tryptic soy agar (TSA) supplemented with nystatin (0.2%) used for mesophilic bacterial population and violet red bile agar (VRBA) for bacteria belonging to the Enterobacteriaceae family (e.g. coliforms – Gram-negative bacteria).

Surface samples from the floor and ventilation grids were collected by swabbing corresponding indoor sites with a 10 cm x 10 cm square stencil, disinfected with a 70% alcohol solution between samplings. In these case, besides the media already applied for air sampling also DG18 was use for fungal contamination characterization. All of the collected samples were incubated at 27 °C for 5–7 days (fungi) or at 30 °C and 35°C for 7 days (mesophilic bacteria and coliforms – Gram-negative bacteria, respectively). After laboratory processing and incubation of the samples, quantitative (colony-forming units—CFU.m-3 and CFU.m-2) results for fungi and bacteria were obtained, with the exception of samples collected from the ventilation grid. In this last case, prevalence was achieved through the isolate number of each species identified. Fungal identification was achieved through macro- and microscopic characteristics, as described by Hoog et al. (2002).

2.2. Particles measurement

Assessment of particulate matter (PM) was conducted with portable direct-reading equipment (Lighthouse, model 3016 IAQ). Particles concentration measurement was performed at five different sizes (PM0.5, PM1, PM2.5, PM5, PM10). Additionally, data related with particle number concentration by each diameter size was also available with the use of this equipment. In this last case, particles results were given in six different diameters sizes, namely; 0.3 μm, 0.5 μm, 1 μm, 2.5 μm, 5 μm and 10 μm. As mentioned in previous studies, this data was also collected because might be more closely correlated with adverse PM health effects (Wichmann et al., 2000; Welgers et al., 2004). Each measurement was done through 5 minutes since it was considered as representative of the working period.

3. Evidence

3.1. Bioaerosols assessment

Concerning fungal load, the indoor air results ranged from 40 to 92 CFU.m-3 not surpassing the WHO reference value for occupational environments (150 CFU.m-3). Outdoor reference sample presented higher fungal than all the indoor samples (152 CFU.m-3) (Figure 1). However, 4 out of 5 indoor samples
presented different fungal species from the ones identified in the outdoor sample, suggesting fungal contamination from within (Kemp et al., 2003). Regarding surfaces, both media applied presented different results. In MEA the results ranged from 0 to 50x103 CFU.m-2 and in three sampling sites were not observed fungal isolates. Regarding DG18 the adequacy for enumerating xerophilic fungi (Siqueira et al., 2011), that can be more present in surfaces samples, justify not only the higher fungal load that ranged from 0 to 11x104 CFU.m-2, but also the fact that only two samples didn’t present fungal grow. In addition, the same media inhibits overgrowth of fast growing fungi such as members of the Mucorales and Trichoderma (Siqueira et al., 2011).

Figure 1 - Fungal load obtained on air samples and surfaces. Dashed line (WHO reference value - 150 CFU/m²)

Regarding ventilation grids only 1 out of 4 samples didn’t showed fungal grow and the fungal load ranged from 0 to 11x104 isolates.
Concerning fungal characterization Cladosporium sp. was the most prevalent in indoor air samples (74.7%) and Aspergillus sp. on surface samples (MEA 100%; DG18 57.1%) (Table 1).

<table>
<thead>
<tr>
<th>Table 1 - Fungal distribution on the samples analysed</th>
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<tbody>
<tr>
<td><strong>Air</strong></td>
</tr>
<tr>
<td>Cladosporium sp.</td>
</tr>
<tr>
<td>Penicillium sp.</td>
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<tr>
<td>Aspergillus sp.</td>
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<tr>
<td>Others</td>
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<th><strong>Surface</strong></th>
<th><strong>Frequency (n; %)</strong></th>
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<tr>
<td><strong>MEA</strong></td>
<td><strong>DG18</strong></td>
</tr>
<tr>
<td>Aspergillus sp.</td>
<td>7000; 100</td>
</tr>
<tr>
<td>Cladosporium sp.</td>
<td>-</td>
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<tr>
<td>Ulocladium sp.</td>
<td>-</td>
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<th><strong>Split</strong></th>
<th><strong>Frequency (n; %)</strong></th>
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<tr>
<td><strong>MEA</strong></td>
<td><strong>DG18</strong></td>
</tr>
<tr>
<td>Chrysosporium sp.</td>
<td>10000; 20</td>
</tr>
<tr>
<td>Penicillium sp.</td>
<td>30000; 60</td>
</tr>
<tr>
<td>Cladosporium sp.</td>
<td>10000; 20</td>
</tr>
<tr>
<td>Aureobasidium sp.</td>
<td>-</td>
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<td>Others</td>
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Besides the presence of Aspergillus section Fumigati (in 3 out of 5 indoor air samples) and A. section Versicolores (1 ventilation grid) requiring implementation of corrective measures according to the American Industrial Hygiene Association (AIHA 1996) in the Field Guide for the Determination of Biological Contaminants in Environmental Samples, we should point out that
most of the species/strains isolated from Aspergillus sections have toxigenic potential (Nielsen, 2003). We should also note that besides fungal higher range on surfaces samples, Aspergillus sections Candidi, Versicolores and Terrei were only isolated on surfaces and were not found on air. This result highlights the need to also collect samples from surfaces besides air, to ensure a more complete fungal contamination assessment (Viegas et al., 2016).

Total bacterial load ranged from 28 CFU.m-3 to 136 CFU.m-3 (Figure 2). Guidelines of some studies (Goyer et al., 2001; Mandal and Brandl, 2011), that suggest 1000 CFU.m-3 for total bacteria were applied to evaluated bacterial burden, since there aren’t national guidelines for imposing limit values for bacterial load. The bacterial load of the outdoor reference sample (136 CFU.m-3) was the same in one indoor sample and higher than all other samples, suggesting bacterial contamination sources from outside, which could be explained by the fact that bacteria are microorganisms found in nature (Terkoda, 1987). Regarding surfaces, for mesophilic bacterial population the results ranged from 14x103 to 48x105 CFU.m-2 and in two sampling sites were not observed isolates, and only one sampling site presented coliforms (Gram-negative bacteria). The presence of Gram-negative bacteria may be due to human activity (Zhu et al., 2003) and could increase the risk for the production of endotoxins which can cause acute and toxic inflammation of the lungs (Miaškiewicz-Peska et al., 2011). In the ventilation grids the range varied between 1 to 4 isolates and only one sample didn’t showed mesophilic bacteria grow, and coliforms were not observed. Microbiota presence in ventilation grids can be due to inadequate maintenance of HVAC systems (Charkowska, 2011).

![Figure 2 - Bacterial load obtained on air samples and surfaces](image)

We should also consider that culture based-methods only give information about a small fraction from the microbiota present and this can lead to several bias concerning occupational exposure assessments as already reported (Viegas et al., 2015). Thus, besides having only a semi-quantitative data concerning exposure to bioaerosols we should bear in mind the impossibility to correlate with workers health complaints.
3.2. **Particles assessment**

In the case of particles data (Figure 3 and 4), results showed that the office 5G3 presented the higher values for PMC and PNC. In the case of PMC, it was the larger particles group (PM10) that had the higher value and, for PNC metric, were the smaller particles (0.3 μm) that presented the higher values. This distribution was similar in all the measurements done. These findings are well in agreement with a previous report published by Fromme (2012) that stated that the particles in indoor environments are composed by larger particles that determine primarily the mass of the environmental aerosol and the particle number concentration (PNC) are dominated almost exclusively by the smaller particles, including ultrafine particles (<100 nm). There is no explanation for the difference obtained in 5G3 since no features were observed during the measurements that could explain this. Even the description of tasks made before the campaign did not mention differences that could explain this. However, the higher results obtained for PM2.5 and PM10, when compared with Portuguese regulation (Portuguese Norm 1796, 2014) are well below the reference values of 10 mg/m3 and 3 mg/m3, respectively. Additionally, the outdoor measurement presented, for both metrics, the higher values demonstrating that outdoor air is the contamination source for the indoor air. Therefore, since the windows are kept close (Fromme, 2012). The outdoor contamination depends of several factors such as traffic, temperature, humidity and much others. However, when comparing the obtained results of PNC in the outdoor measurement with the ones already reported in several European cities, it seems that much higher values were observed during our measuring campaign (Fromme, 2012).

![Figure 3](image1.png)  
**Figure 3** - Results of PMC in each location (mg/m³)

![Figure 4](image2.png)  
**Figure 4** - Results of PNC in each location (counts)
3.3. Conclusions

Results obtained confirm occupational exposure to bioaerosols and particles not related directly with the activity but with the ventilation system. Both risk factors can induce the health symptoms reported by workers.

4. References

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