Biological Air Assessment in Primary Schools - The ARIA Project

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INTRODUCTION: Exposure and sensitization to indoor allergens are important risk factors for asthma and allergic respiratory diseases, playing a key role in triggering and exacerbating allergy and asthma symptoms in children (Salo *et al.*, 2009). While children's greatest exposure to indoor allergens is at home, other public places where they spend a large amount of time, such as primary schools, may also be sources of significant allergen encounters (Abramson *et al.*, 2006), such as bacteria and fungi. Children are considered a susceptible group because they are particularly vulnerable to the development of respiratory diseases, such as asthma, and also spend much of their time inside classrooms, in a confined atmosphere, reasons why they should deserve priority attention in indoor air quality (IAQ) studies. In Portugal, this is an increasing and important subject, proved by the development of studies in this area, more sorely since the publication of the national legislation, Ordinance no. 353-A/2013 of December 4th, that establish reference values of maximum concentration for selected indoor air pollutants. The results of this project are part of an ongoing project (ARIA Project), that intend to study the exposure of children to indoor air in 20 public primary schools and investigate the associated respiratory and allergic related health effects in 1600 children's.

OBJECTIVES: The main goals of this study are 1) characterize IAQ in primary schools, during the winter season, by the evaluation of total bacteria and fungi concentrations in classrooms, in Porto, and compare these results with the current revised national standards; 2) identify the main fungi species found in the evaluated areas; 3) analyze the health impacts caused by biological pollutants on children.

MATERIALS AND METHODS: Biological assessment took place during winter season, as this period provides the worst case scenario of exposure due to the enclosed environments. From January to February 2014, out of a predicted total of 20 primary schools to be study, 6 schools were assessed. A total of 22 classrooms (average of 4 rooms per school) were evaluated regarding their biological contamination during their normal occupancy, through the analysis of total bacteria count, fungi count and identification. Air sampling was carried out with a microbiological air sampler (Merck Air Sampler MAS-100) and using *Tryptic Soy Agar* (TSA) for total bacteria and *Malt Extract Agar* (MEA) for fungi. Outdoor air samples were also collected for comparison to the indoor measurements. Quantification of bacteria and fungi was performed by naked eye count and the identification of fungal colonies was based upon phenotypic characteristics and followed standard mycological procedures, Results were expressed as colony-forming units per cubic meter of air (CFU/m^3) and compared with the recently revised Portuguese standards. Classical statistical methods were used to estimate means, medians and frequencies (percentages).

RESULTS AND DISCUSSION: Mean bacteria concentration is above the reference value for all schools evaluated, from 6 to 548 times higher (Table 1). Regarding primary schools mean fungi concentrations, only in one school the value is according with the reference value, nonetheless being very close to the established limit (Table 1). If the previous Portuguese legislation was still ruling (Decree-Law no 79/2006 of April 4th: Bacteria and Fungi reference value = 500 CFU/m^3), concerning fungi concentrations found indoors, only 2 of the 6 primary schools were above the reference value. The revised legislation changed the limit: for fungi, concentrations indoors shouldn't be higher than outdoors; for bacteria, concentration found outdoors plus 350 CFU/m^3 is the reference value.

Cladosporium sp. and *Penicilium* sp. were the prevalent species found in 5 primary schools, being *Penicillium* sp. the more common species in 4 of these 5 schools. In another school, *Aspergillus fumigatus*, a known potential pathogenic/toxigenic species, was the prevalent specie identified (40%), followed by *Cladosporium* sp. (20%) and *Penicillium* sp. (20%). Nevertheless, this specie was identified in all evaluated primary schools. The presence of toxin-producing fungi like *Aspergillus fumigatus* indoors should be a cause for concern considering the potential risk of mycotoxicosis (Ayanbimpe *et al.*, 2010). Another species from *Aspergillus* genus were found in 2 schools: *Aspergillus flavus* (2% in both schools) and *Aspergillus niger* (2% in both schools).

Table 1 – Biological Air Assessment Descriptive Statistics

	Bacteria	Fungi
	Mean ± Standard Deviation (CFU/m^3)	
PTE01	2466 ± 984	323 ± 58
Outdoor	398	394
Reference *	**748**	**394**
PTE02	1182 ± 407	242 ± 138
Outdoor	98	170
Reference *	**448**	**170**
PTE03	1442 ± 308	542 ± 313
Outdoor	44	122
Reference *	**394**	**122**
PTE04	3855 ± 3241	6393 ± 2830
Outdoor	96	160
Reference *	**446**	**160**
PTE05	2836 ± 1781	363 ± 80
Outdoor	174	192
Reference *	**524**	**192**
PTE06	2194 ± 1036	253 ± 131
Outdoor	4	118
Reference *	**354**	**118**

***** Ordinance no. 353-A/2013 of December 4th: Bacteria _ Indoor < Outdoor + 350 CFU/m^3; Fungi _ Indoor < Outdoor CFU/m^3.

CONCLUSION: The presented results show that 30% of the targeted primary schools raise concern due to the high concentrations of bacteria and fungi in all evaluated areas. Toxin-producing fungi *Aspergillus fumigatus*, identified in all primary schools and in considerable concentrations, is another warning sign of the poor IAQ that exists in classrooms full of young children. This factor, large number of occupants, along with low ventilation rates may be the cause of the results found in this ongoing study. Thus, to promote the air exchange, classrooms should be ventilated, by opening the doors and windows, during the classes' breaks or, when that is not possible, through the implementation of a mechanical ventilation system allowing a minimum flow of fresh air of 24 m^3/hour.person. All the results obtained will contribute to more knowledge on how children response to indoor environmental conditions and how needs and requirements of individuals can be translated to requirements of the indoor environment, both in home and in school.

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