Bioburden in Indoor Environments

Subjects: Environmental Sciences

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The exposure to particles and bioaerosols has been associated with the increase in health e ects in children. The objective of this study was to assess the indoor exposure to bioburden in the indoor microenvironments more frequented by children. Air particulate matter (PM) and settled dust were sampled in 33 dwellings and four schools with a medium volume sampler and with a passive method using electrostatic dust collectors (EDC), respectively. Settled dust collected by EDC was analyzed by culture-based methods (including azole resistance profile) and using qPCR.

Results showed that the PM2.5 and PM10 concentrations in classrooms were higher than in homes and highly exceeded the limit values established by the Portuguese legislation for indoor air quality. The fungal species most commonly found in bedrooms was *Penicillium* sp. (91.79%), whereas, in living rooms, it was *Rhizopus* sp. (37.95%). *Aspergillus* sections with toxigenic potential were found in bedrooms and living rooms and were able to grow on VOR.

indoor air quality microenvironments schools dwellings bioburden electrostatic dust collector

1. Introduction

Children are more susceptible to air pollutants compared to adults since they breathe more air relative to their body weight, their immune system is still in development and they have a lower ability to deal with the toxicity due to their undeveloped airways [1][2]. Children spend more than 85% of their time in indoor environments, mainly at home and school [3] and therefore it is essential to assess the indoor air quality (IAQ) in these microenvironments to estimate their integrated exposure to air pollutants.

Pollutants such as particulate matter (PM) are linked to an increase in morbidity and mortality [4][5]. PM is a complex mixture of small-diameter particles with different physical and chemical characteristics. PM is classified according to their diameter (e.g., PM2.5 and PM10, which are particles with an aerodynamic diameter smaller than 2.5 and 10 µm, respectively), because this physical characteristic highly affects the penetration into the respiratory tract [6][7]. PM2.5 or fine particles reach the lower respiratory tract, while the PM2.5–10 or coarse particles can reach the upper respiratory tract. In addition, the health impact of the PM depends on its composition, which is highly determined by the emission sources.

Bioaerosols are usually defined as PM with biological origins such as microorganisms, pollen and plant fibers. The exposure to biological agents can lead to a wide range of adverse health effects, including allergies, infection diseases, breathing problems and cancer [4].

Previous studies reported a wide range of environmental factors that influence bioburden (covering bacteria and fungi) indoors, such as the occupancy of the spaces [8][9], building layout, ventilation [10] and cleaning procedures including the type of products applied [4]. Furthermore, poor maintenance of heating, ventilation and air conditioning systems can also enhance the hazardous effects of many biological and nonbiological pollutants [11]. Due to the influence of these multiple environmental variables, sampling bioburden should be performed by passive methods, together with more conventional air sampling [12][13][14][15]. Indeed, passive methods allow defining the contamination of a larger period of time (ranging from weeks to several months), whereas air samples can only replicate the load from a shorter period of time (mostly minutes) [16].

The electrostatic dust collector (EDC) is a passive collection device easy-to-use that comprises an electrostatic polypropylene cloth [17]. The use of this device is gradually increasing since it is low-cost and effective for the collection of dust [16][18][19], and it has already been applied for the bioburden assessment in several indoor environments [16][19][20][21][22][23][24][25][26][27].

The emergence worldwide of drug-resistant human pathogenic fungal species, such as *Candida* sp. and *Aspergillus fumigatus*, and the increasing reports of therapeutic failure against fungal infections caused by environmental resistant strains [28][29][30], has revealed the need of surveillance of fungal resistance in the indoor and outdoor environments, which is mostly described for *Aspergillus* section *Fumigati* [31][32][33][34][35][36].

2. Particulate Matter Assessment

The PM2.5 and PM10 average concentrations in the classrooms were 31.15 and 57.83 µg/m^3, respectively, with a range between 19.47 and 52.91 µg/m^3 for PM2.5 and between 32.72 and 109.02 µg/m^3 for PM10. Table 1 shows that in dwellings, the concentrations ranged between 6.05 and 67.96 µg/m^3 for PM2.5 and between 9.14 and 72.95 µg/m^3 for PM10, with an average concentration of 15.26 µg/m^3 and 18.95 µg/m^3, respectively. The PM2.5 concentrations exceeded the 8-hr limit value established by the Portuguese legislation for indoor air quality (Portaria 353-A/2013, 25 µg/m^3) in 50% of the schools and in 12% of the dwellings and the PM10 limit value (50 µg/m^3) was exceeded in 50% of the schools and in 3% of the dwellings.

Table 1. Settled dust (g/m^2/d) and PM2.5 and PM10 concentrations (µg/m^3) measured in dwellings and schools.

		Settled Dust (g/m^2/d)	PM2.5 (µg/m^3)	PM10 (µg/m^3)
Schools	Average	1.42	31.15	57.83
	Range (min–max)	1.28–1.57	19.47–52.91	32.72–109.02

		Settled Dust ($g/m^2/d$)	PM2.5 ($\mu g/m^3$)	PM10 ($\mu g/m^3$)
Dwellings	Average	3.36	-	-
	Range (min–max)	1.27–11.16	-	-
Living Rooms	Average	3.60	15.26	18.95
	Range (min–max)	1.28–11.16	6.05–67.96	9.14–72.95
Bedrooms	Average	3.11	-	-
	Range (min–max)	1.27–10.74	-	-

Regarding the settled dust collected by the EDC, the schools presented an average level of 1.42 $g/m^2/d$ with a range between 1.28 and 1.57 $g/m^2/d$ and the dwellings registered an average of 3.36 $g/m^2/d$ with a range between 1.27 and 11.16 $g/m^2/d$. In dwellings, the living room presented an average amount of 3.6 $g/m^2/d$ and the bedroom of 3.11 $g/m^2/d$ (Table 1).

3. Bacterial Contamination Assessment

From the 31 samples collected in the bedrooms, the total bacteria contamination ranged from below the detection limit to 1.42×10^3 $CFU/m^2/d$, with the Gram-negative bacteria contamination, ranging from below the detection limit to 3.15×10^1 $CFU/m^2/d$.

Total bacteria contamination in the 33 EDC collected in living rooms ranged from below the detection limit to 3.42×10^3 $CFU/m^2/d$, with the Gram-negative bacteria contamination, ranging from below the detection limit to 4.60×10^1 $CFU/m^2/d$.

In the 4 EDC samples collected in the classrooms, the total bacteria contamination ranged from below the detection limit to 6.2×10^1 $CFU/m^2/d$, while there was no contamination by Gram-negative bacteria (Table 2).

Table 2. Bacteria contamination ($CFU/m^2/d$) in each studied location.

Location			Total Bacteria	Gram-Negative Bacteria
	Average	N	$CFU/m^2/d$	$CFU/m^2/d$
Bedrooms	Range (min–max)	31	*–1.42×10^3	*–3.15×10^1
Living Rooms	Range (min–max)	33	*–3.42×10^3	*–4.60×10^1
Classrooms	Range (min–max)	4	*–6.2×10^1	-

4. Fungal Contamination Assessment

A total of 31 EDC were collected from bedrooms. The fungal contamination in these samples ranged from lower the detection limit to 2.00 × 10^3 CFU/m^2/d (D30) in MEA, and from lower the detection limit to 2.81 × 10^3 CFU/m^2/d (D32) in DG18. The most commonly found fungal species in MEA was *Penicillium* sp. (2.00 × 10^3 CFU/m^2/d; 89.43%), followed by *Cladosporium* sp. (1.59 × 10^2 CFU/m^2/d; 7.10%) and *Chrysosporium* sp. (2.56 × 10^1 CFU/m^2/d; 1.14%; Table 3. In DG18, the most prevalent species were *Cladosporium* sp. (2.81 × 10^3 CFU/m^2/d; 90.44%), *Penicillium* sp. (2.07 × 10^2 CFU/m^2/d; 6.67%) and *Aspergillus* sp. (1.05 × 10^2 CFU/m^2/d; 1.23%; Table 3). Four different *Aspergillus* sections were identified in the EDC samples from the bedrooms, two found in MEA (*Nigri* and *Fumigati*; 1.05 × 101 CFU/m^2/d), and two in DG18 (*Candidi* and *Circumdati*; 3.81 × 101 CFU/m^2/d; Figure 1).

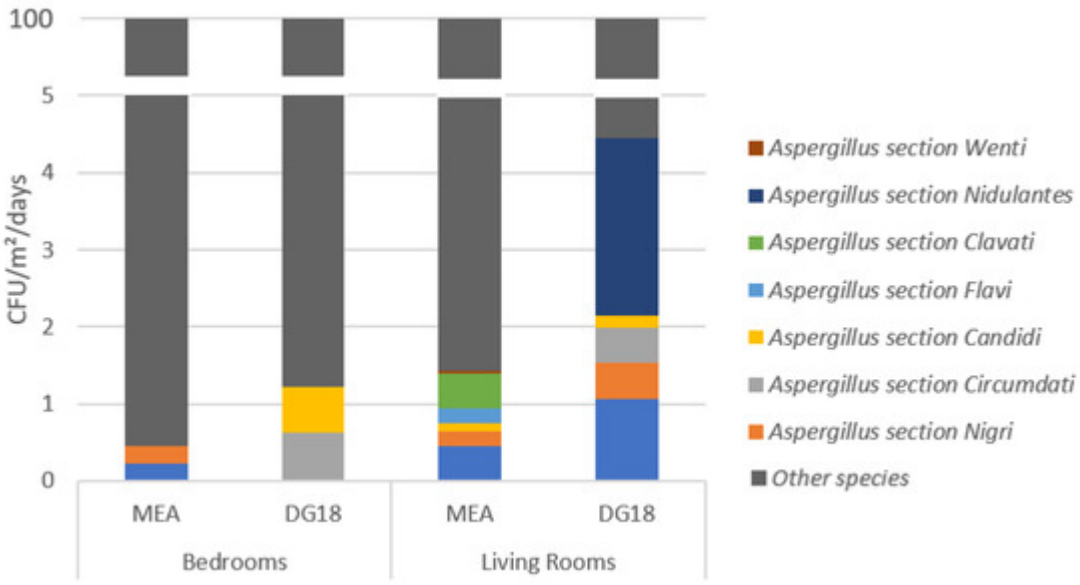

Figure 1. *Aspergillus* sections identified in the electrostatic dust collectors (EDC) samples from the bedrooms and the living rooms.

Table 3. Fungal species found in each studied location.

Location	Genus/Species	MEA			DG18		
		N	CFU/m^2/d	%	N	CFU/m^2/d	%
Bedrooms	*Alternaria* sp.	2	1.05 × 10^1	0.47	1	1.05 × 10^1	0.34
	Aureobasidium sp.	1	5.24 × 10^0	0.23	1	5.24 × 10^0	0.17
	Chrysosporium sp.	3	2.56 × 10^1	1.14	2	9.49 × 10^0	0.31
	Cladosporium sp.	8	1.59 × 10^2	7.10	14	2.81 × 10^3	90.44

Location	Genus/Species	MEA			DG18		
		N	CFU/m^2/d	%	N	CFU/m^2/d	%
	Geotrichum sp.	1	4.14 × 10^0	0.18	1	5.24 × 10^0	0.17
	Penicillium sp.	17	2.00 × 10^3	89.43	12	2.07 × 10^2	6.67
	Aspergillus sp.	2	1.05 × 10^1	0.47	2	3.81 × 10^1	1.23
	Fusarium sp.	2	2.18 × 10^1	0.97	0	*	*
	Crysonilia sitophila	0	*	*	2	2.10 × 10^1	0.68
	Alternaria sp.	1	5.24 × 10^0	0.04	0	*	*
	Aspergillus sp.	2	1.33 × 10^2	0.97	2	1.68 × 10^2	4.91
	Aureobasidium sp.	1	4.91 × 10^0	0.04	0	*	*
Living rooms	*Chrysonilia* sp.	2	5.24 × 10^3	38.11	1	2.62 × 10^3	76.55
	Chrysosporium sp.	4	2.64 × 10^3	19.19	8	6.68 × 10^1	1.95
	Cladosporium sp.	13	2.22 × 10^2	1.61	12	1.7 × 10^2	4.96
	Fusarium sp.	0	*	*	1	2.46 × 10^1	0.72
	Geotrichum sp.	0	*	*	2	1.48 × 10^1	0.43
	Penicillium sp.	14	2.65 × 10^2	1.93	16	3.54 × 10^2	10.33
	Rhizopus sp.	2	5.24 × 10^3	38.11	0	*	*
	Ulocladium sp.	0	*	*	1	5.24 × 10^0	0.15

Location	Genus/Species	MEA			DG18		
		N	CFU/m^2/d	%	N	CFU/m^2/d	%
Classrooms	*Penicillium* sp.	2	1.76 × 10^1	64.21	0	*	*
	Chrysonilia sp.	1	4.91 × 10^0	17.90	1	4.91 × 10^0	19.74
	Cladosporium sp.	1	4.91 × 10^0	17.90	1	4.91 × 10^0	19.74
	Aspergillus sp.	0	*	*	1	4.91 × 10^0	19.74
	Chrysosporium sp.	0	*	*	1	1.02 × 10^1	40.79

In the 33 EDC collected from the living rooms, the fungal contamination ranged from lower the detection limit to 5.24 × 10^3 CFU/m^2/d (D3, D6 and D28) in MEA, and from lower the detection limit to 2.62 × 10^3 CFU/m^2/d (D32). In MEA, the most common was *Rhizopus* sp. (5.24 × 103 CFU/m^2/d; 38.11%), followed by *Chrysonilia* sp. (5.24 × 10^3 CFU/m^2/d; 38.11%) and *Chrysosporium* sp. (2.64 × 10^3 CFU/m^2/d; 19.19%); in DG18, *Chrysonilia* sp. (2.62 × 10^3 CFU/m^2/d; 76.55%), followed by *Penicillium* sp. (3.54 × 10^2 CFU/m^2/d; 10.33%) and *Cladosporium* sp. (1.7 × 10^2 CFU/m^2/d; 4.96%) were the most prevalent (Table 3). A total of eight *Aspergillus* sections were identified in the samples from the living room. Five different sections were found in MEA, including *Aspergillus* section *Fumigati* (6.18 × 101 CFU/m^2/d), *Flavi* and *Nigri* (2.62 × 10^1 CFU/m^2/d; Figure 1). In DG18, six *Aspergillus* sections were identified, with the most prevalent being *Nidulantes* (7.89 × 10^1 CFU/m^2/d), followed by *Fumigati* (3.67 × 10^1 CFU/m^2/d) and *Clavati* (1.57 × 10^1 CFU/m^2/d; Figure 1).

Four EDC were recovered from classrooms. The fungal contamination in the MEA samples ranged from the lower detection limit (S1) to 1.76 × 10^1 CFU/m^2/d (in the three remaining samples), and in DG18 from the lower detection limit (S1 and S3) to 1.02 × 10^1 CFU/m^2/d (in S4). Three different fungal species were identified in the MEA samples: *Penicillium* sp. (1.76 × 10^1 CFU/m^2/d; 64.21%), *Chrysonilia* sp. and *Cladosporium* sp. (4.91 × 10^1 CFU/m^2/d; 17.90%; Table 3). Four fungal species were found in DG18: *Chrysosporium* sp. (1.02 × 10^1 CFU/m^2/d; 40.79%), *Aspergillus* section *Nidulantes*, *Chrysonilia* sp. and *Cladosporium* sp. (1.02 × 10^1 CFU/m^2/d; 19.74%; Table 3).

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