

Indoor pollutant exposure among children with and without asthma in Porto, Portugal, during the cold season

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Abstract Considering the time spent in enclosed spaces, indoor air pollutants are of major interest because of its possible impact on health. However, to date, few studies have analysed the air concentrations of a large set of indoor pollutants of respiratory health relevance in dwellings, particularly in Portugal. This study aimed to measure the concentrations of air pollutants that are present in residential buildings and to investigate whether some clustering pattern of indoor air pollutants exists in the dwellings of children with (case group) and without asthma (control group). Measurements were taken in 30 and 38 dwellings of asthmatic and non-asthmatic schoolchildren, respectively, located in the city of Porto, Portugal, during the cold season (October 2012–April 2013), to assess the concentrations of 12 volatile organic compounds (VOC), aldehydes, PM_{2.5}, PM₁₀, bacteria and fungi. Toluene, d-limonene, formaldehyde, PM_{2.5}, bacteria and fungi are widely present in dwellings, sometimes in relatively high concentrations in reference to WHO guideline values. Moreover, concentrations of CO₂ exceeding 1000 ppm were often encountered, indicating that 70 % of all dwellings had poor ventilation (<4 L/s person). While exposures to common dwelling indoor pollutants are similar for schoolchildren with and without asthma, except for d-limonene levels, the

identification and control of VOC and PM sources is important and prudent, especially for vulnerable individuals.

Keywords Volatile organic compounds · PM_{2.5} · PM₁₀ · Dwellings · Indoor exposure · Asthma

Introduction

Asthma and allergies are an important public health issue worldwide (Rufo et al. 2015a). The most recent revised global estimate of asthma suggests that as many as 334 million people have asthma and 14 % of the world's children experience asthma symptoms (Mallol et al. 2013). The prevalence of asthma in 6- to 7-year-old children in Western Europe ranges from 8.3 % for girls to 10.9 % for boys (Mallol et al. 2013). In Portugal, Madureira et al. (2015a) reported that among 8- to 10-year-old children, the prevalence of wheeze in last year was 8.3 % for girls and 14.3 % for boys in 2014, which was similar to the European mean values (Delmas et al. 2010).

Many risk factors regarding respiratory problems have been largely investigated and are well known, such as gender (Delmas et al. 2010) or family heritability (Cookson et al. 2011). However, some environmental exposures are suspected of playing a key role in the onset/development of these diseases, such as particulate matter (PM) from outdoor air pollution, as reported by Clark et al. (2010). However, an increasing number of research studies, focused on indoor air pollutants, have also been carried out (Annesi-Maesano et al. 2013; Madureira et al. 2012, 2014, 2015b). Taking into account the time spent by children in enclosed indoor spaces, the rising tightness of buildings associated with reduced air circulation and the use of new substances in building construction materials, furniture and consumer products, the indoor

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environment is of major concern (Heinrich 2011; Hulin et al. 2012; Madureira et al. 2016).

Volatile organic compounds (VOC) are very commonly found in dwellings, as many indoor materials and utilities used indoors contain VOC (Godish 2001), and have already been linked to asthma and other respiratory problems (Rive et al. 2013; Rufo et al. 2015a, b; Rumchev et al. 2004). A similar relationship was reported regarding aldehydes and the development and exacerbation of asthma and allergies (Annesi-Maesano et al. 2012; McGwin et al. 2010; Rumchev et al. 2002). Among indoor air pollutants, PM is also very commonly found indoors (Breysse et al. 2005). PM could penetrate from the outside (e.g. traffic, industrial emissions) and some is generated and remains indoors (e.g. smoking, cooking and cleaning) (Massey et al. 2012; Simoni et al. 2010; Weichenthal et al. 2007). PM_{2.5} (particles with the diameter smaller than 2.5 µm) often contains hazardous substances and, when compared with coarse PM, penetrates deeper into the respiratory system (Chithra and Nagendra 2012; Grigg 2009; Massey et al. 2012). Besides VOC, aldehydes and PM, indoor airborne bacteria and fungi have become a matter of concern also due to associated respiratory symptoms, allergies and asthma, and the perturbation of the immunological system (Mendell et al. 2011; WHO 2009b). While indoor sources of airborne bacteria include the presence of humans, pets, soils and plants (Bowers et al. 2012; Womack et al. 2010), the sources for indoor airborne fungi can come from outdoor air and indoor reservoirs (Crawford et al. 2015; WHO 2009b). The complexity of bacterial and fungal indoor exposure (spatial variability, indoor sources, infiltrations from outdoor emissions, seasonal variability) suggests the need to further characterize their presence.

However, in this growing body of evidence, to date, few studies have simultaneously assessed the air concentrations of a large set of indoor pollutants of respiratory health relevance in dwellings, particularly in Portugal. In this context, the aims of this study were to measure the concentrations of VOC, aldehydes, PM_{2.5}, PM₁₀, bacteria and fungi in Portuguese residential buildings and to investigate whether some clustering pattern of indoor air pollutants exist in dwellings of schoolchildren with and without asthma.

Material and methods

Study design and dwelling selection

This study is a part of a large investigation on the impact of the indoor environment on asthma and allergy among children in Porto, Portugal (Madureira et al. 2015c). The first step was a cross-sectional investigation in 20 schools. Depending on the size of the school, two to four classrooms per school were selected, in a total of 73 classrooms. The preference was for

classrooms with high-density occupation as well as full weekly occupation time by the same class, and, if possible, at different floor levels. At this step, a modified International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire was given to the parents of all 1639 children, between the ages 8–10. Responses for 1099 children were received (participation rate, 69 %) (Madureira et al. 2015c).

The present study is the second step including 38 dwellings from asthmatic children (cases) and 30 dwellings from non-symptomatic children (controls) randomly recruited among those who took part in the first step (cross-sectional study in schools). Asthmatic cases were selected on the basis of an affirmative response to one of the following questions in the ISAAC questionnaire: “Has your child ever had asthma diagnosed by a doctor?” and “In the past 12 months, has your child had wheezing or whistling in the chest?”. Controls were selected among those children whose parents/legal guardians had answered “no” to both questions. In both groups, cases were excluded whenever homes had recently been rebuilt/refurbished (<6 months) or a change of residence had occurred since completing the questionnaire. The 68 dwellings were investigated between October 2012 and April 2013 during the cold season. Visits included a walk-through survey of building characteristics and chemical, physical and biological air sampling (described below). The study was conducted respecting the Declaration of Helsinki and was approved by the Ethics Committee of the University of Porto (22/CEUP/2011). Written informed consent was obtained from parents or legal guardians of the children.

Walk-through survey and checklist

A walk-through survey and detailed checklist were completed by a researcher in each dwelling to describe the outdoor environment, general information about the building (period of construction and renovation, surface, number of rooms), specific information about the child’s bedroom (floor, wall and ceiling materials, the presence of pressed-wood furniture, heating and ventilation systems) and daily living habits in general and specifically during the sampling period (heating, aeration, cooking, number of occupants and pets, specific activities or use of specific products). The checklist was defined according to the literature about the determinants of the measured pollutants and based on similar studies carried out previously (Csobod et al. 2014; Dassonville et al. 2009; Roda et al. 2011).

Air sampling collection and analysis

The current study measured major health-related air pollutants (WHO 2010). Measurements of VOC, aldehydes, PM_{2.5}, PM₁₀, bacteria, fungi, carbon dioxide (CO₂), temperature and relative humidity levels were conducted simultaneously

both indoors and outdoors. The children's bedroom was chosen since it represents the room with the longest exposure time in their dwellings. Indoor samples were collected from the rear of the bedroom, 1–1.5 m above the floor (breathing zone) and not closer than 1 m from a wall, a door or an active heating system under normal conditions regarding heating and airing of the dwelling as well as the use of rooms (ISO 16000-1 2004). In the cold season, the windows were usually closed due to outdoor weather conditions and/or due to the fact that heating systems were turned on. Outdoor sampling sites were constrained by access to electricity and whenever possible not closer than 1 m from the building at heights of 1–2 m above the ground. All the outdoor samplers and equipment were mounted in a shelter protected from direct sunlight and precipitation.

The parents were requested to fill in a weekly time activity diary reporting their personal activities, including smoking, cooking and cleaning activities, ventilation habits, number of occupants, etc. Moreover, the families were asked to maintain their regular routine during the environmental monitoring.

VOC and aldehydes were continuously collected during 7 days using commercially available passive samplers (stainless-steel sampling tubes containing Tenax® TA (60/80 mesh) for VOC and Radiello® passive devices (RAD 165, Sigma-Aldrich) for aldehydes. Tenax tubes were then thermally desorbed (Dani STD 33.50) and quantified using a non-polar column by gas chromatography (Agilent Technologies 6890N) coupled to a mass spectrometry detector (Agilent Technologies 5973), following the method described by ISO 16000, part 6 (2011). Total VOC (TVOC) concentration was quantified using the toluene response factor, and concentrations were calculated as the sum of VOC eluting between hexane and hexadecane (included), expressed as toluene. To control contamination during transport and sampling, a field blank was employed in every dwelling. All samples were taken in duplicate to verify the reproducibility of measurements. Formaldehyde and acetaldehyde concentrations were determined using isocratic reverse phase high-performance liquid chromatography with a UV detector operated at 360 nm, according to ISO 16000-4 (2011). Aldehydes were identified and quantified by comparison of their retention times and peak areas with those of standard solutions. Each cartridge was sealed after sampling and brought back to the laboratory where it was stored in a refrigerator (<4 °C). As an internal quality control, duplicate samplings were collected. Field blanks were collected and analysed to assess possible contamination through the sample collection and analysis process. Portable TSI DustTrak DRX photometers (model 8533; TSI Inc.) were used for the assessment of PM_{2.5} and PM₁₀ concentrations. This equipment measures particles with a laser photometer based on light scattering principle. The measuring range of the equipment is $1\text{--}150 \times 10^3 \mu\text{g}/\text{m}^3$ with accuracy of $\pm 0.1\%$ of reading of $1 \mu\text{g}/\text{m}^3$. The equipment operates with a

flow rate of 3.0 L/min using a built-in diaphragm pump powered by an internal battery. Instruments were set to continuously measure for at least 8 h; while the logging intervals were set to 1 min between each sample. The monitors are zeroed automatically using the external zeroing module, in order to minimize the zero drift effect. Meanwhile, the photometers were calibrated externally once per year at the factory. Bacterial and fungal air samples were obtained using a single stage microbiological air impactor (AirIdeal™, bioMérieux SA) according to the NIOSH Method 0800 (1998) and European Standards (2000). Tryptic Soy Agar (TSA) (supplemented with 0.25 % cycloheximide) and Malt Extract Agar (MEA) (supplemented with 1 % of chloramphenicol) were used as culture media for bacteria and fungi, respectively. These are general growth media that facilitate non-selective growth. Air was drawn through the impactor at 100 L/min, and sequential duplicate air samples (duplicates of 100 and 250 L) were collected both indoors and outdoors. The detailed sampling and analytical method has previously been described by Madureira et al. (2015c). Carbon dioxide, temperature and relative humidity levels were measured, concurrently with the other air parameters (both indoors and outdoors), for 7 days every 5 min (IAQ-CALC monitor 7545, TSI). The ventilation rate was calculated from monitored CO₂ concentrations using the decay method described by Ramalho et al. (2013).

Statistical analysis

A descriptive statistics analysis was performed to characterize the indoor air quality (IAQ) at residential buildings. The Shapiro-Wilk test was used for normality testing. The distribution of all indoor air parameters was skewed; thus, they were described by median, 25th percentile (P25) and 75th percentile (P75). In addition, mean, standard deviation (SD), minimum and maximum were also presented. Volatile organic compound data were explored to determine the proportion of samples above the detection limit. Concentrations of VOC below the detection limits were excluded from the statistical analyses. The 7-day means of all measured parameters concentrations were used in the analyses. Statistical analysis was performed using SPSS Statistics version 19 (SPSS Inc., Released 2009, USA). A *p* value below 0.05 was considered statistically significant.

Results

The proportion of boys is significantly higher in the case group than in control group; however, the remaining characteristics (age, BMI, family history of allergic disorders, number of siblings and parental education level) showed no significant differences (Table 1). In data not shown, 26 (70 %) of

Table 1 Demographic and health data of schoolchildren with and without asthma

	Asthmatic children, <i>n</i> = 38		Non-asthmatic children, <i>n</i> = 30		<i>p</i> value
	<i>n</i>	%	<i>n</i>	%	
Sex					0.006
Girls	9	23.7	17	56.7	
Boys	29	76.3	13	43.3	
Age (years) ^{a, b}	8.5 (0.7)		8.6 (0.7)		0.782
Body mass index (kg/m ²) ^{a, b}	17.7 (2.01)		18.1 (2.20)		0.422
Family history of allergic disorders					0.280
No	14	36.8	15	50.0	
Yes	24	63.2	15	50.0	
More than 3 siblings ^b					0.305
None	6	17.6	6	24.0	
1	14	41.2	12	48.0	
2 or more	14	41.2	7	28.0	
Mothers' education level					0.827
0–6 years	3	7.9	3	10.0	
7–9 years	7	18.4	3	10.0	
10–12 years	7	18.4	7	23.3	
≥ 13 years	21	55.3	17	56.7	0.777
Fathers' education level ^b					
0–6 years	5	13.5	4	14.3	
7–9 years	7	18.9	6	21.4	
10–12 years	12	32.4	9	32.1	
≥ 13 years	13	35.1	9	32.1	
Asthma-like					
Ever wheeze	33	89.2	5	17.2	<0.001
Wheeze (<30 days)	12	31.6	0	0	0.001
Asthma in school	7	18.9	0	0	0.009
Rhinitis-like					
Ever runny/blocked nose ^c	26	68.4	9	30.0	0.001
Nasal allergy (<12 months) ^c	26	68.4	8	26.7	0.002
Eye irritation (<12 months) ^c	21	55.3	2	6.7	<0.001
Ever nasal allergy	17	47.2	5	16.7	0.023
Doctor-diagnosed nasal allergy ^b	13	36.1	4	13.3	0.025
Skin diseases					
Ever itchy rash (for 6 months)	12	32.4	10	33.3	0.926
Ever eczema	10	30.3	8	26.7	0.633
Other respiratory diseases apart from cold ^b					
Dry cough at night (<12 months)	30	83.3	7	23.3	<0.001
Cough episodes	9	25.0	2	6.7	0.036
Phlegm episodes	9	24.3	1	3.3	0.013
Skin ^b					
Hand rash	7	19.4	4	13.8	0.549
Face rash	7	18.9	4	13.8	0.582
Eczema	1	3.1	4	14.3	0.122
Eye ^b					
Eye irritation	16	44.4	7	24.1	0.091
Swollen eye	12	33.3	1	3.6	0.004
Nose ^b					
Runny nose	28	73.7	13	44.8	0.017
Blocked nose	32	88.9	13	43.3	<0.001
Lower airways					
Dry throat ^b	16	44.4	9	30.0	0.232
Sore throat ^b	21	60.0	14	46.7	0.286
Irritating cough	29	76.3	9	30.0	<0.001
Shortness of breath ^b	21	56.8	2	6.7	<0.001
Systemic ^b					
Headache	16	44.4	10	33.3	0.361
Symptom(s) improve on returning home ^b	3	10.3	5	22.7	0.233

^a Mean (standard deviation)^b Sample size for each variable may vary due to missing data^c Not occurring due to cold

cases had been diagnosed with asthma by a physician. As expected, the prevalence of symptoms of rhinitis and other respiratory diseases apart from cold were higher among cases, but no significant differences were found regarding skin diseases. Table 1 also presents the prevalence of recent symptoms and diseases (<3 months) by case and control groups. In general, prevalence of skin and systemic symptoms was similar in both groups. Moreover, the reported frequency of swollen eye, runny and blocked nose, irritating cough and shortness of breath was significantly higher in the case group.

Main characteristics of the dwellings

A summary of the characteristics of the dwellings investigated by case and control group is presented in Table 2. There were no significant differences in potential sources of pollutants measured, except for a few characteristics. Most of the dwellings were apartments (>80 %), and the number of inhabitants varied between 2 and 7. Regarding the building period, in general, case group children lived in older dwellings (mean = 40 years); whilst controls often lived in more recent houses (mean = 24 years). There were no significant differences in the proximity of potential outdoor air pollution sources (e.g. attached garage, highway, power plant for the building, industry) in the homes of asthmatic children when compared with control children, except for gasoline dispensing facilities ($p = 0.020$). However, more than three-quarters of the dwellings were located near the streets with busy traffic at least one part of the day, i.e. rush hour (78 % of asthmatic children's homes and 77 % of non-asthmatic children's homes; $p = 0.915$); 89 % of case homes and 80 % of control homes were in front of a car park. Only 13.2 % of the case families had air conditioning. The most common home heating sources were gas and electricity, with some homes having multiple heating sources. Other potential sources of indoor air pollution identified were fireplaces (three dwellings from case group and three dwellings from control group) and attached garage (64 and 53 % in case and control homes). In addition, more common visible signs of dampness (such as visible mould growth, damp spots) than mouldy odours were reported by the research team, but bubbles or yellow discoloration were not observed in any of the homes. Signs of indoor mould growth were observed mostly in the case group homes (47 vs. 20 %, $p < 0.022$). Signs of high humidity (dampness/condensation on windows) were more common in case homes (51 % in case homes vs. 37 % in control homes, $p = 0.233$). Likewise, the characteristics were similar in the bedrooms of asthmatic and control children. In the present study, significant differences were found in the living conditions such as the presence of air conditioner ($p = 0.040$), water leakage and visible mould in the previous year ($p = 0.025$) and plywood ($p = 0.007$) between the case and control groups. The

living conditions of the families were also summarized in Table 2.

VOC, aldehydes, PM_{2.5}, PM₁₀, bacteria and fungi concentrations

Table 3 summarizes the descriptive data for the indoor measured parameters. Valid data on indoor VOC were missing in one case and five control homes due to problems in the laboratory analysis. In the present study, four target VOC were observed in less than 25 % of samples [benzene, tetrachloroethylene (T4CE), naphthalene and styrene]; while trichloroethylene (T3CE) was not detected in any sample. For both groups, the highest indoor median concentration was found for toluene, d-limonene, formaldehyde and acetaldehyde whereas lowest median levels were measured for benzene and o-xylene. Overall, there were no significant differences in concentrations of indoor air parameters in the dwellings of asthmatic children compared to those of non-asthmatic children, except for d-limonene levels (median = 10.6 $\mu\text{g}/\text{m}^3$ in case homes vs. median = 15.6 $\mu\text{g}/\text{m}^3$ in control homes, $p = 0.013$). The median concentration of TVOC was 97.4 $\mu\text{g}/\text{m}^3$ with a maximum of 793.6 $\mu\text{g}/\text{m}^3$ measured in a case home; while in control homes, the median TVOC levels were 125.0 $\mu\text{g}/\text{m}^3$.

Aldehydes were detected in 100 % of the dwellings (Table 3). Levels of formaldehyde were below the current indoor exposure guidelines of 100 $\mu\text{g}/\text{m}^3$ (WHO 2010). No significant difference was observed between case and control homes.

Measurements on PM_{2.5} and PM₁₀ could be carried out in 12 homes from the case group and 18 homes from the control group due to pump technical problems in the measurement equipment during field campaign. Median concentrations of PM_{2.5} and PM₁₀ were lower in the homes of children with asthma (54 vs. 67 $\mu\text{g}/\text{m}^3$, $p = 0.966$ for PM_{2.5}; and 56 vs. 71 $\mu\text{g}/\text{m}^3$, $p = 0.966$ for PM₁₀).

Furthermore, bacteria concentrations in the dwellings of asthmatic children varied widely from 98 to 6528 CFU/ m^3 (median = 774 CFU/ m^3); while in the control homes, it varied from 188 to 6528 CFU/ m^3 (median = 652 CFU/ m^3); however, there were no statistically significant differences ($p = 0.608$). Concerning fungi levels, higher indoor levels were observed in the control group (case group vs. control group: 170 vs. 301 CFU/ m^3 ; $p = 0.253$).

CO₂ levels and ventilation rate

Data on indoor CO₂ were missing in 11 case homes and six control homes due to non-acquisition of information by the equipment during the sampling period. Around 61 % of the measured bedrooms had 7-day median CO₂ concentration above 1000 ppm, approximately 4 % of the rooms had an

Table 2 Building characteristics and living conditions of schoolchildren with and without asthma

	Case, <i>n</i> = 38 <i>n</i> (%)	Control, <i>n</i> = 30 <i>n</i> (%)	<i>p</i> value
Building characteristics			
Type of building			0.655
Single-family house	4 (10.5)	4 (13.3)	
Semi-detached house	2 (5.3)	2 (6.7)	
Apartment	32 (84.2)	24 (80.0)	
Building age ^{a, b}	34.0 (40.7) ^c	23.8 (14.4) ^c	0.799
Building location	25 (65.8)	23 (76.7)	0.601
Urban area	12 (31.6)	7 (23.3)	
Sub-urban area	1 (2.6)	0	
Rural area			
Street with heavy traffic (<200 m) ^b			1.00
No	6 (16.7)	5 (16.7)	
Yes	30 (83.3)	25 (83.3)	
Near of source of outdoor air pollution ^b			
Car park	32 (88.9)	24 (80.0)	0.320
Busy road (at least part of the day)	28 (77.8)	23 (76.7)	0.915
Highway	5 (13.9)	5 (16.7)	0.756
Power plant for the building	1 (2.8)	0	0.361
Other power plant (up to 1 km)	6 (16.7)	1 (3.3)	0.082
Gasoline dispense facilities	6 (16.7)	0	0.020
Industry (up to 10 km)	35 (97.2)	30 (100)	0.361
Agricultural sources (up to 3 km)	2 (5.6)	2 (6.7)	0.852
Attached garage	23 (63.9)	16 (53.3)	0.389
Air conditioner ^a			0.040
No	33 (86.8)	30 (100.0)	
Yes	5 (13.2)	0	
Water leakage/damage indoors (<12 months) ^b			0.025
No	36 (100)	26 (86.7)	
Yes	0	4 (13.3)	
Visible mould growth ^b			0.022
No	19 (52.8)	24 (80.0)	
Yes	17 (47.2)	6 (20.0)	
Bubbles or yellow discoloration ^b			1.00
No	36 (100)	30 (100)	
Yes	0	0	
Dampness/condensation on the lower part of the windows (during winter) ^{a, b}			0.233
No	18 (48.6)	19 (63.3)	
Yes	19 (51.4)	11 (36.7)	
Noticeable mould odour ^b			0.400
No	33 (91.7)	29 (96.7)	
Yes	3 (8.3)	1 (3.3)	
Child's bedroom characteristics			
Bedroom area (m ²) ^c	11.5 (2.67)	12.0 (3.33)	0.193
Heating system ^b			0.287
No	26 (72.2)	25 (83.3)	
Yes	10 (27.8)	5 (16.7)	
Type of floor material ^b			0.933
Synthetic smooth (PVC/Vinyl, linoleum)	1 (2.8)	1 (3.3)	

Table 2 (continued)

	Case, <i>n</i> = 38 <i>n</i> (%)	Control, <i>n</i> = 30 <i>n</i> (%)	<i>p</i> value
Laminate parquetry	29 (80.6)	24 (80.0)	
Stone/ceramic tiles	4 (11.1)	1 (3.3)	
Wood/cork	2 (5.6)	4 (13.3)	
Carpet	0	0	
Furniture materials ^b			
Wood	27 (77.1)	25 (83.3)	0.537
Plywood	21 (60.0)	8 (26.7)	0.007
Textiles	0	1 (3.3)	0.280
Metal	2 (5.7)	2 (6.7)	0.874
Plastic laminate or composite	1 (2.9)	0	0.355
Water leakage/damage indoors (<12 months) ^b			0.277
No	34 (94.4)	26 (86.7)	
Yes	2 (5.6)	4 (13.3)	
Visible mould growth ^b			0.239
No	27 (75.0)	26 (86.7)	
Yes	9 (25.0)	4 (13.3)	
Visible damp spots on walls, ceiling or floor ^b			0.466
No	26 (72.2)	24 (80.0)	
Yes	10 (27.8)	6 (20.0)	
Bubbles or yellow discoloration ^b			0.273
No	36 (100.0)	29 (96.7)	
Yes	0	1 (3.3)	
Noticeable mould odour ^b			0.668
No	34 (94.4)	29 (96.7)	
Yes	2 (5.6)	1 (3.3)	
Presence of carpets ^b			0.134
No	13 (38.2)	6 (20.7)	
Yes	21 (61.8)	23 (79.3)	
Stuffed toys ^b			0.062
No	15 (42.9)	6 (20.7)	
Yes	20 (57.1)	23 (79.3)	
Living conditions			
Domestic pets (with fur)			0.502
No	27 (71.1)	19 (63.3)	
Yes	11 (28.9)	11 (36.7)	
Exposure to tobacco smoke at home ^b	16 (44.4)	11 (39.3)	0.681
Number of smokers at home ^{a, b}			0.245
None	22 (59.5)	15 (50.0)	
One	12 (32.4)	8 (26.7)	
Two or more	3 (8.1)	7 (23.3)	
Number of cigarettes smoked at home (inside spaces) ^{a, b}			0.257
None	27 (73.0)	17 (58.6)	
1 or 2	4 (10.8)	3 (10.3)	
3 to 4	1 (2.7)	5 (17.2)	
5 to 10	3 (8.1)	3 (10.3)	
11 to 20	1 (2.7)	1 (3.4)	
More than 20	1 (2.7)	0	
Use of air fresheners (frequently/often)			0.401
No	27 (71.1)	24 (80.0)	

Table 2 (continued)

	Case, <i>n</i> = 38 <i>n</i> (%)	Control, <i>n</i> = 30 <i>n</i> (%)	<i>p</i> value
Yes	11 (28.9)	6 (20.0)	0.066
Use of incense sticks (frequently/often) ^{a, b}			
No	22 (57.9)	23 (79.3)	0.158
Yes	16 (42.1)	6 (20.7)	
Presence/use of humidifier ^a			0.158
No	34 (89.5)	23 (76.7)	
Yes	4 (10.5)	7 (23.3)	

^a Data provided from parents' questionnaire^b Sample size for each variable may vary due to missing data^c Mean (standard deviation)

average CO₂ concentration exceeding 2000 ppm although none of the rooms exceeded 3000 ppm. These numbers could actually be higher if it is taken into account that 59 % of case family dwellings and 63 % of control family homes did not comply the 1000 ppm as a reference value. The aforementioned results may imply that the derived median ventilation rate was low in both groups (2.08 L/s per person vs. 2.60 L/s per person; *p* = 0.126), showing the presence of some closed dwellings. Almost 70 % of all dwellings had a ventilation rate below 4 L/s per person.

Temperature and relative humidity levels

Median indoor temperature was significantly lower in the bedrooms of asthmatic children than in the bedrooms of non-asthmatic children (16.7 vs. 17.7 °C; *p* = 0.045). Approximately 93 % of houses (35 case and 28 control homes) had at least one average temperature and/or relative humidity which fell outside the recommended range. The recommended temperature should be between 20 and 24 °C in winter. In around 93 % of the measurements, the temperature was under 20 °C and no values were observed over 26 °C. The indoor relative humidity should be between 30 and 70 %. Regarding relative humidity of the indoor air, none of the values were below 30 %. However, it was observed that in 32 % of the homes, the relative humidity value was over 70 %. There were a significant number of homes (97 %) with relative humidity over 50 %.

Discussion

Main findings

For the current study, dwellings of 68 children (38 asthmatics and 30 non-asthmatics) were investigated for a large set of respiratory health relevant indoor pollutants (VOC, among which aldehydes, PM_{2.5}, PM₁₀, bacteria and fungi) since

residential IAQ is not regulated, and the levels of indoor pollution are not widely known.

In the present study, benzene, T3CE, T4CE, naphthalene and styrene were detected in less than 25 % of the samples, and many VOC were found in nearly every home in both groups, including toluene and other aromatics, which are commonly present in household products, paints adhesives, synthetic fragrances, vehicle emissions and many others; and d-limonene, which is a constituent of cleaning products, air fresheners and fragrances (Nazaroff and Weschler 2004; Steinemann et al. 2011). In both groups toluene, d-limonene, formaldehyde and acetaldehyde have the highest median concentrations indoors. Rive et al. (2013) showed that the dwellings of asthmatics were more polluted by benzene than those of the non-asthmatics. Similar to the present study, a previous case-control study including 150 asthmatics and 150 non-asthmatic children showed no differences in indoor air pollution among the two groups (Diette et al. 2007). The results from the present study suggest that differences in lifestyle could influence IAQ, while it is possible that individuals with asthma and their families may selectively adapt their behaviour to avoid certain environmental triggers as part of a multifaceted approach to control asthma. It would be reasonable to expect that families with asthmatic children clean their homes more frequently than control families, a fact that could lead to higher indoor levels of terpenes such as d-limonene in case homes. In this study, no significant statistical difference was found in what regards cleaning frequency in both the case and control homes; suggesting that, although the families of both groups clean their homes with a similar frequency, the case families probably use low-emitting consumer products. This fact might explain the statistically significant differences found in indoor d-limonene concentrations between case and control group; with higher levels of d-limonene found in the control group. Another possible explanation might be that the homes of asthmatic children have higher flow rates of outdoor air than of control homes. Furthermore, it must be underlined that in the present study, no statistically significant differences were found in the living

Table 3 Summary statistics of indoor VOCs, aldehydes, PM_{2.5}, PM₁₀, bacteria, fungi, CO₂, temperature and relative humidity levels in the bedroom of the study dwellings (October 2012–April 2013)

	Case, <i>n</i> = 38							Control, <i>n</i> = 30							<i>p</i> value
	<i>n</i>	<i>n</i> > DL ^a	Mean	SD	Median	P25–P75	Min–Max	<i>n</i>	<i>n</i> > DL ^b	Mean	SD	Median	P25–P75	Min–Max	
Benzene, µg/m ³	37	5	1.12	0.12	1.06	1.02–1.25	1.01–1.28	25	3	4.72	3.33	4.97	1.27–7.92	1.27–7.92	0.746
Toluene, µg/m ³	37	36	25.9	69.0	5.92	3.99–12.8	1.01–398.5	25	25	19.6	34.0	8.03	4.96–17.1	1.64–167.4	0.225
m/p-xylene, µg/m ³	37	36	8.70	15.8	3.34	2.05–7.66	1.03–86.3	25	22	11.6	21.3	4.90	2.66–9.37	1.01–89.8	0.586
o-xylene, µg/m ³	37	31	4.18	5.54	2.30	1.80–3.75	1.06–29.2	25	21	5.44	8.19	2.51	2.00–4.48	1.23–30.4	0.500
d-limonene, µg/m ³	37	34	22.6	38.2	10.6	4.58–23.6	1.11–177.0	25	25	49.0	67.6	15.6	8.26–67.9	1.46–278.2	0.013
α-pinene, µg/m ³	37	29	6.59	10.4	3.05	2.02–5.55	1.15–54.1	25	19	3.65	2.96	2.65	1.77–4.50	1.04–14.2	0.651
T3CE, µg/m ³	37	–	–	–	–	–	–	25	–	–	–	–	–	–	–
T4CE, µg/m ³	37	5	1.77	0.34	1.92	1.43–2.04	1.25–2.11	25	4	6.71	8.08	3.40	1.52–15.2	1.47–18.6	0.929
Naphthalene, µg/m ³	37	6	3.69	5.74	1.47	1.09–5.08	1.09–15.4	25	7	17.9	33.1	1.69	1.17–27.2	1.06–89.7	0.062
Styrene, µg/m ³	37	6	1.78	0.73	1.41	1.25–2.57	1.24–2.96	25	2	1.24	0.10	1.24	1.17–1.30	1.17–1.30	0.441
TVOC, µg/m ³	37	37	146.7	157.9	97.4	55.1–149.5	24.1–793.6	25	25	203.5	210.4	125.0	63.8–260.0	18.0–895.2	0.236
Formaldehyde, µg/m ³	38	38	14.6	10.4	11.4	6.90–17.5	3.68–50.7	30	30	16.6	9.49	14.8	8.53–22.2	5.22–43.3	0.199
Acetaldehyde, µg/m ³	38	38	11.5	8.94	8.69	6.43–14.6	2.15–52.1	30	30	15.0	17.4	10.9	6.62–14.5	2.87–85.2	0.459
PM _{2.5} , µg/m ³	12	–	89	76	54	42–123	36–287	18	–	79	64	67	46–87	19–307	0.966
PM ₁₀ , µg/m ³	12	–	92	78	56	46–131	38–296	18	–	82	64	71	48–90	20–308	0.966
Bacteria, CFU/m ³	38	–	1542	1904	774	369–1723	98–6528	30	–	1413	1878	652	336–1585	188–6528	0.608
Fungi, CFU/m ³	38	–	1038	1837	170	96–1006	34–6528	30	–	524	869	301	181–513	66–4805	0.253
CO ₂ , ppm	27	–	1176	361	1121	828–1463	697–1913	24	–	1260	514	1157	810–1594	671–2641	0.865
Ventilation rate, l/s per person	35	–	4.05	4.04	2.60	1.66–5.02	0–18.2	28	–	3.35	4.28	2.08	1.17–4.20	0–20.5	0.126
Temperature, °C	38	–	16.7	2.28	16.7	15.2–18.1	10.7–22.3	30	–	17.6	1.71	17.7	16.2–18.5	14.6–23.0	0.045
Relative humidity, %	38	–	64	10	65	57–71	36–84	30	–	66	8	66	60–72	43–82	0.436

DL detection limit; SD standard deviation; P25 25th percentile; P75 75th percentile; Min minimum; Max maximum; T3CE trichloroethylene; T4CE tetrachloroethylene; TVOC total volatile organic compounds

^a Number of homes with values above the detection limit

conditions between the case and control groups, such as the presence of pets, use of air fresheners, incense stick, humidifiers, stuffed toys and smoking habits at home. Moreover, although statistical significant differences were observed for the presence of plywood and air conditioner between both groups, special caution should be taken into account based on the small and unbalanced number of cases in both groups.

In the present study, the median concentrations of TVOC were lower than $200 \mu\text{g}/\text{m}^3$, with a total of four homes (two homes for each group) exceeding $600 \mu\text{g}/\text{m}^3$. These results are higher than those exposure levels reported by Rumchev et al. (2004) (median of $78.5 \mu\text{g}/\text{m}^3$ in case homes and $36.2 \mu\text{g}/\text{m}^3$ in control homes), who also reported that children exposed to TVOC at levels higher than $60 \mu\text{g}/\text{m}^3$ are four times more likely to have asthma than those who were not exposed to such levels. In addition to the VOC indoor sources, the proximity of heavy traffic roads as well as of gasoline dispensing facilities and the existence of attached garages could also contribute to the indoor TVOC concentrations; although there were no significant differences among those variables in the homes of asthmatic children when compared with control children, except for those homes close to gasoline dispensing facilities ($p = 0.020$). The furniture age was not taken into consideration although it may have some influence on the VOC emissions, decreasing over time; and on consequent health effects.

Formaldehyde and acetaldehyde were detected in every indoor sample. Measured levels of indoor formaldehyde are not strictly comparable with IAQ WHO guidelines (2010) because of differing average exposure times; however, it is reasonable to assume that they did not exceed WHO for neither the case or control homes. It is likely that indoor formaldehyde concentrations are derived from indoor sources such as gas appliances, building materials including new coverings, smoking and the use of air fresheners and, ultimately influenced by ventilation rates. Acetaldehyde, emitted from building materials or combustion sources such as cigarette smoke, was also to be higher for control rather than case homes, even at low levels; however, the differences were not statistically significant.

Concerning PM, the number of samples does not equal 68 in total due to equipment problems. Therefore, the results have to be carefully analysed. WHO air quality guidelines 24 h values for $\text{PM}_{2.5}$ were exceeded in the majority of homes (100 % in case group and 89 % in control group) (WHO 2005); while 20 homes (eight case and 12 control homes) exceeded the corresponding value PM_{10} guidelines value ($50 \mu\text{g}/\text{m}^3$). As reported elsewhere, smoking is a major determinant of indoor PM concentrations (Fontham et al. 2009; WHO 2009c). Previous studies have consistently reported increased PM concentrations in homes with smokers, indicating that smoking contribution to indoor $\text{PM}_{2.5}$ ranges from 25 to $45 \mu\text{g}/\text{m}^3$. Nonetheless, as the sampling of $\text{PM}_{2.5}$ and PM_{10}

was performed in the children bedroom, the results might be not so influenced by tobacco smoke, although more than 25 % of the parents in both groups referred exposure to tobacco smoke at home. In addition, some specifically identified sources of PM (e.g. traffic emissions, combustion for heating and/or cooking), and human-related activities (e.g. burning candles, incense, cleaning) can contribute to the increase of indoor PM levels (Chao and Cheng 2002) in both groups.

There are also many other potential indoor exposures including bacteria and fungi agents. Regarding bacteria levels, it was found in higher concentrations indoors than outdoors; while the similarity of indoor and outdoor airborne fungal concentrations suggests limited indoor sources. Although without significant statistical differences, higher concentrations of fungi were observed in the control group than in the case group homes. Only median bacteria concentration in asthmatic and non-asthmatic homes exceeded the value recommended by Portuguese legislation ($500 \text{ CFU}/\text{m}^3$) (Decree Law No. 79/2006 2006). The high indoor concentration of bacteria may derive from several factors, including levels in outdoor environment, from the human self-activities, such as breathing, sweating and movement causing particle re-suspension (WHO 2009b). Low ventilation rates and crowded conditions increase CO_2 levels and bioeffluent concentrations, including bacteria and fungi. In addition, building characteristics and activities might reflect the respective higher levels. Previously, large concentrations of fungi and bacteria in association with visible mould growth have been reported, but some studies have found no difference in indoor concentrations between houses with mould problems and “reference” houses (WHO 2009a). Suggested reasons for a lack of association in some studies included the variability of indoor sources including occupant behaviour, cleaning activities, etc. (WHO 2009a). In the present study, during the house surveys, signs of indoor mould growth were mostly observed in the case group homes (47 vs. 20 %, $p < 0.022$); signs of high humidity were observed in 51 % of the case homes and 37 % of the control homes ($p = 0.233$). In addition, all microbiological samples were collected with bedroom windows closed. Supporting these results are the more common signs of high humidity (dampness/condensation on windows) in the case homes (51 % of case homes). It should be noted that microbiological agents were determined using short-term indoor measurements and thus require careful and caution interpretation given the influence of outdoor concentrations and the great variation that can occur very rapidly (Chew et al. 2003; Cho et al. 2000). In addition, the culture-based method has some limitations as stated above. Furthermore, the non-identification of the species specific may contribute to the lack of association between microbiological agents’ exposure and health outcomes. Species level identification and molecular quantification methods may alleviate some of the inconsistencies.

With regard to indoor temperature, the differences may be associated with building characteristics (e.g. less exposed façades, sharing internal walls in the case of apartments), but also with occupant behaviour (e.g., different strategies for heating systems/apparatus on/off, and for ventilation).

Comparison with previous studies

In general, VOC concentrations are lower than levels reported in other studies in homes of individuals with asthma (or with asthma-like symptoms). For instance, in Sweden, toluene averaged $120 \mu\text{g}/\text{m}^3$ (range from 1 to $2330 \mu\text{g}/\text{m}^3$) in living rooms and bedrooms sampled in 1991 and 1992 (Norbäck et al. 1995); and in Korea, toluene, p/m-xylene had mean concentrations of $31 \mu\text{g}/\text{m}^3$, in the homes of children sampled in 2008 (Hwang et al. 2011). Regarding a recent Canadian study (Zhu et al. 2013), median concentrations were comparable for benzene, toluene, xylenes, and limonene. However, a slightly higher concentration for α -pinene was found. Concentrations of VOC measured in different studies can differ for many reasons. Levels in more recent studies (after 2000 or so) are often lower due to progress made in controlling indoor and outdoor emissions, as well as the reduction or elimination of indoor smoking. Because outdoor VOC levels provide a “baseline,” indoor concentrations can be affected by urbanization, proximity to industry, traffic and other emission sources and meteorology. Regional differences in building design (including attached garages), building materials, and climate can influence both ventilation rates and emissions.

In the present study, in both groups, the concentration of formaldehyde is somewhat lower compared with previous studies. Indoor levels of formaldehyde in dwellings generally range between 7.3 and $50.4 \mu\text{g}/\text{m}^3$, although concentrations exceeding $120 \mu\text{g}/\text{m}^3$ have also been observed (Lovreglio et al. 2009). A recent study measured formaldehyde concentrations using passive samplers in residential environments in 12 European cities, found a mean level of $23.8 \mu\text{g}/\text{m}^3$ (Bruinen et al. 2008), which is higher than those in the present study. Dassonville et al. (2009) measured five aldehydes in the bedrooms of 196 Parisian infants and reported a mean concentration of $19.4 \mu\text{g}/\text{m}^3$.

Strengths and limitations

A particular strength of the current study is the wide range of respiratory health relevance indoor pollutants that enhanced the understanding of the indoor air environment in which children, in particular those with asthma, live. Moreover, these results are the first reported in Portuguese dwellings, and are also valuable given the paucity of existing data. Despite a single visit in each dwelling and an indoor assessment limited to 7 days, this study highlights the ubiquitous contamination of the indoor home environment and the occurrence of some

stability is expected because indoor source activities patterns tend to be consistent from day to day taking into account that the participant families were asked not to modify their usual habits and activities and data collection were avoided during vacations, and that a “classic” week was chosen.

Regarding the limitations, this study was based on a small sample size and therefore further exploration in future studies is needed in a larger sample size. In addition, due to small number of houses included in both groups, this study cannot perform a detailed analysis regarding the influence of outdoor air pollution and the impact of building characteristics (e.g. age of furniture, presence of plywood and air conditioner, water leakage and visible mould) on indoor pollutants levels. Limitations also include that housing factors and lifestyle behaviours may have different effects on indoor air pollution in home in different populations, geographic locations and seasons that may affect generalizability. Some potential selection biases were identified (for participating children/families). Briefly, since the present study examined the risk factors for health, it is more likely that families with knowledge or most interested participated in the study. Moreover, as the recruitment of cases and controls was based on volunteer householders, this could have attracted volunteers who were aware of indoor pollution and this may lead to an underestimation of indoor air pollution levels in homes and may have contributed to the lower concentrations observed. A further source of selection bias in this study may have arisen from refusals. Even if the parents gave reasons for their refusals to participate (“too busy”), the refusal of having a survey in a home may be associated with a particular lifestyle, which may affect the concentrations of indoor pollutants. Study children in this investigation may therefore represent a group with relatively lower exposure to indoor air pollutants. Misclassification of controls is also acknowledged in the present study. This is a common potential problem in studies of asthma in children of this age group as they often have the disease without having received a diagnosis. Moreover, questionnaire responses were also subject to bias the results. Recall bias may have been introduced because parents who live with asthmatic/allergic children may be more prone to reporting their exposure, even though information was collected in a uniform manner using a standardized questionnaire. For instance, parents of children with asthma often have some knowledge of environmental triggers and appropriate environmental modifications; therefore, they might be eager to give the “right” answer rather than the true answer, especially to sensitive questions about smoking and having pets. On the other hand, underreporting may also occur among the asthmatic cases because of parental avoidance of reporting their children’s illness. Such reporting bias does not occur to the same extent in longitudinal studies, which make results less subject to recall bias and allow for the assessment of temporal relationships (Morton et al. 2006). Further extended studies would allow the increasing

participation rate among case and control groups and should use shorter questionnaires administered to those who refused to participate in the study in order to analyse how representative the refusals were from the overall study population.

Conclusions

This study provides information regarding VOC, aldehydes, PM_{2.5}, PM₁₀, bacteria and fungi concentration in dwellings of a susceptible population. While evidence supporting the role of key indoor exposures in asthma exacerbation is limited, this study identifies that toluene, d-limonene, formaldehyde, PM_{2.5}, PM₁₀, bacteria and fungi are widely present in dwellings, sometimes in relatively high concentrations in reference to guideline values that have been issued by different bodies, such as WHO. However, this study has shown that there were no significant differences in indoor air parameters in the homes of asthmatic children compared to those homes of non-asthmatic children, except for d-limonene levels. These results suggest that differences in lifestyle could influence indoor air pollutants levels, while it is possible that individuals with asthma and their families may selectively adapt their behaviour to avoid certain environmental triggers as part of a multifaceted approach to control asthma.

Based on these data, it will be possible to develop additional extensive studies on housing and children respiratory outcomes. Moreover, the data collected through the checklist are currently being processed to elaborate a model whose aim will be to estimate indoor air concentrations using qualitative/quantitative characteristics of larger samples of dwellings and of occupants that can be collected through checklist surveys. In conjunction with other studies, this work may lead to dwelling recommendations that modify indoor exposure, based on source control strategies, and positively impact children health, especially in asthmatic children. The results may also help housing designers, builders and home residents to improve IAQ by means of appropriate building materials, clean household products and proper life styles and behaviours.

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Compliance with ethical standards The study was conducted respecting the Declaration of Helsinki and was approved by the Ethics Committee of the University of Porto (22/CEUP/2011). Written informed consent was obtained from parents or legal guardians of the children.

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