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Indoor air quality in Portuguese schools: levels and sources of pollutants

Abstract Indoor air quality (IAQ) parameters in 73 primary classrooms in Porto were examined for the purpose of assessing levels of volatile organic compounds (VOCs), aldehydes, particulate matter, ventilation rates and bioaerosols within and between schools, and potential sources. Levels of VOCs, aldehydes, $PM_{2.5}$, PM_{10}, bacteria and fungi, carbon dioxide (CO_2), carbon monoxide, temperature and relative humidity were measured indoors and outdoors and a walkthrough survey was performed concurrently. Ventilation rates were derived from CO_2 and occupancy data. Concentrations of CO_2 exceeding 1000 ppm were often encountered, indicating poor ventilation. Most VOCs had low concentrations (median of individual species <5 $\mu g/m^3$) and were below the respective WHO guidelines. Concentrations of particulate matter and culturable bacteria were frequently higher than guidelines/reference values. The variability of VOCs, aldehydes, bioaerosol concentrations, and CO_2 levels between schools exceeded the variability within schools. These findings indicate that IAQ problems may persist in classrooms where pollutant sources exist and classrooms are poorly ventilated; source control strategies (related to building location, occupant behavior, maintenance/cleaning activities) are deemed to be the most reliable for the prevention of adverse health consequences in children in schools.

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Practical Implications

This study provides quantitative assessment for a large set of indoor air quality (IAQ) parameters in public primary classrooms together with information regarding potential building-wide contamination sources. Elevated levels of CO_2, $PM_{2.5}$, PM_{10} and bacteria may be associated with outdoor pollution sources, occupant behavior, maintenance/cleaning activities and poor ventilation. IAQ investigations often include air sampling, which must be carefully conducted if representative data are to be collected. To better understand sampling results, investigators need to account for the variability of contaminants both within and between schools. These findings are of relevance to public health due to the very large population of exposed school children, especially since attendance in primary schools is compulsory and asthma and allergy are very common diseases in childhood. These data may be useful for assessing health effects of exposure, for understanding the underlying mechanisms and for implementing preventive policies in terms of standards and guidelines.

Introduction

Children spend most of their time indoors, mainly at home and in school. They are particularly susceptible to indoor air pollution when compared to adults due to their under-developed immune and respiratory systems and high inhalation rates per body mass (Viegi et al., 2004). Indoor air pollution is determined by a combination of pollution sources associated with the place, the climate and the culture, i.e. the surrounding ambient air, the building's physical characteristics and the indoor activities (Oliveira Fernandes et al., 2008; Stranger et al., 2007).

In primary schools, IAQ is characterized by a broad array of various indoor pollutants such as volatile organic compounds (VOCs), aldehydes, particulate matter ($PM_{2.5}$ and PM_{10}), moulds, and bacteria (Madureira et al., 2009, 2012, 2014; Mendell and Heath, 2005). Poor IAQ in classrooms can lead to health problems for occupants in addition to reducing learning performance, the attendance of students, and ambient comfort (Annesi-Maesano et al., 2013; Hulin et al., 2010; Mendell et al., 2013; Simoni et al., 2010). Several studies have shown that carbon dioxide (CO_2) concentrations in schools often do not meet building standards due to inadequate ventilation (Annesi-Maesano et al., 2013; Madureira et al., 2009; Simoni et al., 2010). It has been hypothesized that the associations between ventilation levels and health or performance result from the fact that ventilation does not only affect the level of indoor CO_2 but also levels of other pollutants in the indoor environment that are able to cause adverse effects.

Children spend up to 10 h/day in primary schools. Understanding the air pollution in these environments, documenting their concentrations and determining which factors influence these levels is very important. Despite the large population of primary schoolchildren, only a few studies regarding IAQ in Portuguese primary schools have been undertaken (Fraga et al., 2008; Madureira et al., 2009; Martins et al., 2012; Pegas, 2012). Often a specific pollutant, e.g. particulate matter or bioaerosols, or a limited combination of pollutants, is addressed. Just a few studies have investigated the contribution of outdoor or indoor sources for a wide range of IAQ parameters and in a large sample size of classrooms. In addition, the effects of school building/classroom characteristics and occupant behavior on indoor air have not been discussed.

The main purposes of this work were: (i) to assess IAQ parameters in public primary schools located in Porto and to compare the measured indoor concentrations with those published in previous studies and with current guidelines; (ii) to study potential sources of indoor pollutant levels, such as building/classroom characteristics and occupant behavior; and (iii) to assess the variability in pollutant levels within and between schools.

Materials and methods

School buildings and classroom selection

Indoor and outdoor measurements were conducted in 20 public primary schools. All of the schools were located in Porto, the second largest city in Portugal, located in the north of the country (41.16°N, 8.62°W). Depending on the size of the school, two to four classrooms per school, comprised of children aged 8 to 10 years old, were simultaneously investigated. 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. As a result, a total of 73 classrooms were selected. The walkthrough survey and the IAQ sampling in each school occurred within the same visitation period during the winter seasons, from November to March, during the years 2011–2013.

Walkthrough survey and checklist

A walkthrough survey and checklist were completed for each school and indoor space to document information about these parameters and conditions: outdoor environment; building construction, age and size; number of floors; number of occupants; finishing materials; heating and ventilation systems; past occurrences and current visible problems; and potential indoor sources. Classroom characteristics, such as the area, floor, walls, and ceiling conditions, windows, classroom materials (paintings, glues, etc.), maintenance routines and cleaning procedures were also registered, as well as the materials of classroom furniture, the presence of chalkboards, copiers, and plants. The research team asked the school's staff to maintain the same cleaning practices during the study.

IAQ sampling and analysis

Sampling included VOCs, aldehydes, $PM_{2.5}$, PM_{10}, bacteria, fungi, CO_2, carbon monoxide (CO), temperature and relative humidity in each classroom and outdoors. Monitoring was conducted in occupied classrooms during regular daily activities and under representative conditions of occupancy and use of the classrooms.

Safe and childproof sampling locations were selected according to the standard ISO 16000-1 (2004). The instruments were placed on a flat surface, with a height of 1–1.5 m to simulate the primary school children's breathing zone. The sampling locations were no closer than 1 m to any walls, doors or active heating equipment. Furthermore, the indoor sampling locations were selected to be as far away as possible from blackboards, where applicable. Outdoor air samples were taken at places with electricity and a tamper-free environment. The samplers were mounted in a shelter protected from direct sunlight and precipitation, at heights of 1–2 m above the ground, leaving the sampling inlets/sensors unobstructed in order not to compromise the quality of the sampling.

Volatile organic compounds were collected passively onto thermally desorbed adsorbents (Tenax® TA, Sigma-Aldrich, Sintra, Portugal) over five consecutive days (school week, from Monday morning until Friday afternoon). After sampling, the Tenax tubes were thermally desorbed (Dani STD 33.50) and the samples were quantified by gas chromatography (6890N; Agilent Technologies, Santa Clara, CA, USA) coupled to a mass spectrometry detector (GC-MS) (5973; Agilent Technologies), according to the standard ISO 16000-6 (2011). Total VOC 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 equivalents. Laboratory and field blanks, collected in each school, showed concentrations below method detection limits in all cases. All samples were collected in duplicate to verify the reproducibility of measurements. The limits of detection were 1.3 μg/m^3 for toluene, 1.8 μg/m^3 for p-xylene, 2.8 μg/m^3 for d-limonene, 2.5 μg/m^3 for tetrachloroethylene (T4CE), and 4.4 μg/m^3 for naphthalene.

Aldehydes (formaldehyde and acetaldehyde) were sampled by Radiello® passive devices (RAD 165; Sigma Aldrich) during a school week (from Monday morning until Friday afternoon) and determined using isocratic reverse phase high performance liquid chromatography (HPLC) (1220 Infinity LC; Agilent Technologies) with a UV detector operated at 360 nm, according to the standard ISO 16000-4 (2011). Aldehydes were identified and quantified by comparing their retention times and peak areas with those of standard solutions. As an internal quality control, duplicate samplings were collected in one school per each three. Field blanks were collected and analysed to assess possible contamination through the sample collection and analysis process. The detection limits were 0.075 μg/m^3 for formaldehyde and 0.178 μg/m^3 for acetaldehyde.

Portable TSI DustTrak DRX photometers (model 8533; TSI Inc., Shoreview, MN, USA) were used for the assessment of $PM_{2.5}$ and PM_{10} concentrations. This equipment measures particles with a laser photometer, based on the light scattering principle. 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 installed inside each classroom and were set to continuously measure during at least one school day, i.e. for 8.5 consecutive hours. Logging intervals were set to 1 min between each sample. The photometers were calibrated once per year at the factory. As a consequence of the limited number of sampling units, indoor and outdoor particulate matter could not be sampled in parallel and was collected indoors and outdoors sequentially avoiding Monday and Friday weekdays.

Bacterial and fungal air samples were obtained using a single-stage microbiological air impactor (AirIdeal™; bioMérieux SA, Marcy l'Etoile, France), according to the NIOSH method 0800 (1998) and EN 13098:2000 (2000). Tryptic soy agar (supplemented with 0.25% cicloheximide) and malt extract agar (supplemented with 1% of chloramphenicol) were used as culture media for bacteria and fungi respectively. Air was drawn through the sampler at 100 l/min, and sequential duplicate air samples of 100 and 250 l were collected indoors (8 to 16 air samples/school, depending of the number of classrooms) and outdoors (four outdoor samples/school). The air sample collection included daytime sampling, starting at 10.00 a.m., and was conducted discretely to minimize nuisance to normal occupant activities. For each sampling day, agar media blanks per culture media were taken into the field. The air sampler was always cleaned between sample collections with cotton wipes wetted with isopropyl alcohol. After sampling, the agar media plates were transported to the laboratory in a thermal bag for incubation. To quantify the bacterial and fungal concentrations, samples were incubated at 37 ± 1°C for 48 ± 3 h and at 25 ± 3°C for 72 ± 3 h respectively (EN 13098, 2000). Quantification of bacteria and fungi levels was performed by naked eye count in accordance to the methodologies expressed in ISO 4833: 2013 (2013) and EN 13098: 2000 (2000). The quantification limit was established as 10 CFU per plate.

Carbon dioxide, CO, temperature, and relative humidity levels were recorded concurrently with the other air parameters (both indoors and outdoors) during 8.5 consecutive hours per day over a 5-day period (from Monday morning to Friday afternoon). These parameters were measured using an IAQ-CALC monitor (model 7545, TSI, Inc.), which combined an infrared non-dispersive sensor for CO_2, an electrochemical sensor for CO, a thermistor for measuring temperature, and a thin-film capacitive sensor for relative humidity. Measurements were conducted with a time step of 5 min during the investigation week. Calibration was performed once per year at the factory according to manufacturer's specifications.

The ventilation rate for each classroom was estimated based on decay of the indoor CO_2 concentration (emitted by the occupants during the class periods) where the room was precisely documented as non-occupied. On average, only the most trustworthy fractions of the 12-h period (typically the time period matched between 8.00 p.m. and 8.00 a.m.) of CO_2 data extracted for each day was used for further data treatment. The estimated final ventilation rate in the classroom is the time-weighted average of the ventilation rates obtained during the school week after the school was closed and the classroom was empty. The ventilation rate values were estimated using the average outdoor CO_2 concentration over the week measurements period according to ASTM E 741-00 (2006) and ASTM D 6245-98 (2002).

Statistical analysis

The Shapiro-Wilk test was used for normality testing. The distribution of all IAQ parameters was skewed. All data reported in this work regarding $PM_{2.5}$, PM_{10}, CO_2, CO, temperature and relative humidity levels are restricted to periods of actual classroom occupation excluding breaks, lunch time, and periods when the students were elsewhere (e.g. gym). Night-time periods are also excluded to ensure that the sample is representative of the exposure time of the children. Data for individual VOC, total VOCs and aldehydes, measured in parallel and continuously from Monday morning to Friday afternoon (~104 h consecutive hours) were reported for the school week under observation. For VOCs and aldehydes, the number of observations above the method detection limit was calculated. Spearman's rank correlation coefficient was used to realize statistical dependence between VOCs, aldehydes, CO_2, $PM_{2.5}$, PM_{10}, bioaerosols, temperature, and relative humidity.

Within- and between-school variability was evaluated using mixed linear models.

To investigate the relationship between building/classroom characteristics, occupant behavior and IAQ parameters, principal components analysis (PCA) with varimax rotation was applied, as a first approach, to understand how the indoor air parameters were aggregated. The Scree Plot criterion was used to determine the number of components retained. If the factor loading was 0.40 or higher (in absolute value), an item was considered in the indicator. Considering the asymmetry in the distributions of the input variables, we applied a logarithmic transformation to each of the IAQ parameters. After choosing indicators that represented each factor, multiple linear regression was used to assess the factor associated with each variable. The stepwise forward method was used to assess which factors were associated with each input variable (data not shown). In a second approach, a multilevel linear regression with two levels - classroom and school (random effect) - was used to determine which factors explained each input variable and to evaluate the aggregation within schools. The aggregation was estimated using Intraclass Correlation Coefficient (ICC). Four models of multilevel analysis were considered: the first concerned the characteristics of the classroom; the second was represented by characteristics of the school; the third model considered characteristics both of the classroom and school for each indicator; and the fourth and final model is represented by the totality of the classroom and school features, that had a significant effect on the levels of each of the IAQ parameter in order to summarize the effect of all variables resulting from each of IAQ parameters analyzed individually. Statistical analysis was performed using the software R, and multilevel analysis was implemented using the function lme (linear mixed effects) in the nlme library. Statistical significance was defined as $P < 0.05$.

Results

Characteristics of school buildings and classrooms

The mean building age of the schools was 51 years (range: 22–73 years). One-quarter of the school buildings were refurbished before 2004, and 75% were refurbished between 2004 and 2008. All buildings had undergone at least one refurbishment since their construction. Smoking was not legally allowed in any indoor location of any school building (Lei n.° 37/2007).

Table S1 provides the main characteristics of the 73 classrooms. No classrooms had mechanical ventilation systems; opening windows was the only way to ventilate the classroom. In the winter season, the windows were usually closed due to the outdoor weather conditions or due to the fact that heating systems were turned on. All classrooms were cleaned daily with a broom or, less commonly, a vacuum cleaner at the end of the classes. The classrooms were occupied from Monday to Friday, from 9.00 a.m. to 5.30 p.m., with a morning and an afternoon break, between 10.30–11.00 a.m. and 3.30–4.00 p.m. respectively. The lunch break was from noon until 1.15 p.m.

IAQ parameters concentrations and correlations

Volatile organic compounds and aldehydes were detected in almost all indoor samples: benzene, naphthalene, and styrene were detected in less than 25% of classrooms; formaldehyde and acetaldehyde were detected in all samples. Most of the individual VOCs had median levels lower than 5 $\mu g/m^3$. The most abundant VOC in schools were d-limonene (23 $\mu g/m^3$), followed by toluene (6.4 $\mu g/m^3$) (Table 1). As expected, indoor concentrations usually exceeded outdoor levels, although the differences were statistically significant only for d-limonene ($P = 0.001$) (Table S2). Concentrations of toluene, o-xylene, m/p-xylene, d-limonene, and α-pinene across the 73 classrooms were significantly correlated ($\rho = 0.40–0.48$) with total VOCs (Table 2). Benzene was also positively, but not significantly, correlated with total VOCs level. Moreover, strong and significant correlations were found between toluene and m/p-xylene ($\rho = 0.824$, $P < 0.05$) and between m/p-xylene and o-xylene ($\rho = 0.801$, $P < 0.05$). Median outdoor concentrations of benzene and toluene were 2.2 $\mu g/m^3$ and 4.1 $\mu g/m^3$, respectively, reflecting the urban areas sampled. The high indoor/outdoor ratios (I/O > 6) for d-limonene, formaldehyde, and acetaldehyde, and the moderate I/O ratio (~2) for total VOCs and toluene suggest that indoor sources are the main origin for these VOCs. In contrast, the I/O ratio for benzene (0.84) indicates that outdoor sources were the primary contributor for this species.

Classrooms with graphic art activities (e.g., painting) had some of the highest levels measured for certain VOCs (e.g., toluene and naphthalene). Whilst levels of most VOCs appear to be higher in classrooms where art activities were performed, this result should be interpreted cautiously since there were the only two classrooms with graphic art activities during the sampling and they belonged to the same school.

Table 1 Summary statistics of VOCs, aldehydes, particulate matter, bioaerosols, CO_2, CO, temperature, and relative humidity levels

	Location	*n* > MDL[a]	Median (P25-P75)	Mean (s.d.)	Range
Benzene, $\mu g/m^3$	Indoor	7	2.5 (1.6–2.6)	2.2 (0.5)	1.5–2.7
	Outdoor	2	2.2 (1.6–2.8)	2.2 (0.9)	1.6–2.8
Toluene, $\mu g/m^3$	Indoor	72	6.4 (4.5–10.4)	15.1 (34.5)	1.8–202.5
	Outdoor	20	4.1 (2.8–7.2)	5.0 (3.0)	1.2–10.4
m/p-xylene, $\mu g/m^3$	Indoor	71	5.0 (3.3–6.8)	17.7 (59.0)	1.2–365.2
	Outdoor	18	3.3 (1.8–6.4)	4.8 (5.7)	1.1–26.3
o-xylene, $\mu g/m^3$	Indoor	68	2.3 (1.8–3.4)	3.9 (6.9)	1.1–52.4
	Outdoor	15	2.2 (1.9–2.7)	2.7 (2.3)	1.1–10.9
d-limonene, $\mu g/m^3$	Indoor	71	23.1 (11.5–48.6)	38.1 (44.5)	2.8–215.3
	Outdoor	5	2.1 (1.7–2.56)	2.1 (0.5)	1.4–2.6
α-pinene, $\mu g/m^3$	Indoor	63	1.8 (1.4–2.8)	3.4 (5.5)	1.0–32.0
	Outdoor	3	2.3 (1.4–4.1)	2.6 (1.4)	1.4–4.1
Styrene, $\mu g/m^3$	Indoor	13	1.2 (1.2–1.4)	1.4 (0.5)	1.0–2.7
	Outdoor	1	1.0	1.0	–
Total VOC, $\mu g/m^3$	Indoor	73	140.3 (85.5–198.4)	172.2 (145.2)	8.9–820.2
	Outdoor	20	48.2 (35.4–62.9)	54.5 (42.2)	12.1–216
Formaldehyde, $\mu g/m^3$	Indoor	73	17.5 (13.8–23.1)	19.8 (10.9)	8.24–126.9
	Outdoor	19[b]	2.74 (2.27–3.60)	2.90 (0.74)	1.82–4.17
Acetaldehyde, $\mu g/m^3$	Indoor	73	7.65 (4.96–10.4)	9.31 (7.82)	1.92–64.6
	Outdoor	14[b]	0.84 (0.82–1.36)	0.96 (0.58)	0.19–2.09
$PM_{2.5}$, $\mu g/m^3$	Indoor	73	82 (67–106)	94 (40)	39–244
	Outdoor	20	71 (40–100)	81 (61)	27–270
PM_{10}, $\mu g/m^3$	Indoor	73	127 (109–167)	139 (49)	56–320
	Outdoor	20	75 (45–112)	88 (64)	30–276
Bacteria, CFU/m^3	Indoor	73	3200 (1800–5400)	3600 (2300)	200–8400
	Outdoor	20	200 (80–900)	600 (800)	20–3700
Fungi, CFU/m^3	Indoor	73	240 (170–400)	300 (250)	60–1300
	Outdoor	20	200 (120–300)	200 (130)	50–600
CO_2, ppm	Indoor	73	1469 (1195–2104)	1669 (601)	829–3111
	Outdoor	20	442 (364–504)	449 (90)	349–636
CO, mg/m^3	Indoor	73	0.38 (0.07–0.68)	0.48 (0.44)	0.01–1.70
	Outdoor	20	0.22 (0.04–0.55)	0.39 (0.45)	0.01–1.30
Temperature, °C	Indoor	73	20.8 (19.2–21.7)	20.5 (2.06)	14.3–24.6
	Outdoor	20	14.5 (11.7–16.9)	14.6 (3.25)	10.0–20.6
Relative humidity, %	Indoor	73	54 (50–65)	55 (10)	34–74
	Outdoor	20	59 (53–68)	59 (10)	40–75
Ventilation rate, l/s per person	Indoor	73	0.33 (0.21–0.78)	0.87 (1.38)	0.11–7.21

MDL, Method Detection limit; s.d., Standard deviation; P25, 25th percentile; P75, 75th percentile; VOCs, volatile organic compounds.

[a] Number of classrooms (indoor) or schools (outdoor) with values above the method detection limit.

[b] Total of 19 of 20 schools was assessed.

The lowest aldehyde levels were observed for acetaldehyde (Table 1). The median values of formaldehyde levels were lower than the guidelines values established by the WHO (2010) and the EU-INDEX project (Kotzias et al., 2005). However, levels were significantly higher than those measured outdoors (18 vs. 2.7 $\mu g/m^3$, $P < 0.05$).

The indoor median concentration of $PM_{2.5}$ and PM_{10} in all of the classrooms exceeded the 25 $\mu g/m^3$ and 50 $\mu g/m^3$ guideline values suggested by World Health Organization (2010) for a sampling period of 24 h. Whilst for $PM_{2.5}$ there was no significant difference between the levels measured outdoors and inside the classrooms (71 vs. 82 $\mu g/m^3$, $P = 0.098$); for PM_{10} there was a statistically significant difference (75 vs. 127 $\mu g/m^3$, $P = 0.001$). Indoor concentrations exceeded outdoor levels, indicating an I/O ratio higher than the unity, which suggests possible indoor sources (Table S2).

Bacterial concentrations varied widely. Classrooms had a median concentration of bacteria higher than 1000 CFU/m^3 and, in some cases, indoor levels were higher than 3000 CFU/m^3 (Table 1). There were significant differences between indoor and outdoor levels of bacteria, with indoors being significantly higher ($P < 0.05$) (Table S2). Indoor bacterial concentrations were positively and significantly correlated with CO_2 ($\rho = 0.257$; $P < 0.05$) (Table 2), possibly reflecting poor ventilation in classrooms with higher bacterial concentrations. This is supported by the fact that no significant correlations were found between indoor bacteria concentrations and the density of occupation ($\rho = 0.219$; $P > 0.05$). There were no significant differences between classroom and outdoor levels of fungi ($P = 0.066$) (Table S2). In 43% of the classrooms, fungal levels were above specifications in Portuguese legislation ('indoor<outdoor') (Ordinance 353-A/2013). Table 2 showed that only d-limonene was negatively correlated with fungal concentrations, possibly reflecting the use of cleaning products in rooms with higher levels of fungi.

Carbon dioxide levels ranged widely and, among the 73 classrooms surveyed, 86% of the classrooms ($n = 63$) had median CO_2 concentrations exceeding 1000 ppm (ASHRAE 62-2001, 2001). The CO_2 levels changes in the classroom throughout the day and, depending on the occupancy and ventilation, following a path that is theoretically predictable for both the CO_2 accumulation in the room during the time of teaching and for the CO_2 reduction during the breaks. In the present study, CO_2 levels exceeded 1000 ppm during 70% of the occupation measurement time. Maximum CO_2 levels should be interpreted cautiously as they may reflect events such as occupants clustering around and/or breathing on the sensor during occupancy. As expected, indoor CO_2 levels were significantly higher than outdoor levels ($P < 0.05$) with an I/O ratio higher than 3 (Table S2). Higher values were measured in classrooms with higher occupancy density for the longest teaching periods between breaks.

Across the 73 classrooms, the median temperature was 20.8°C, being within the range 20–23°C for 47% of the classrooms. While 38% of the classrooms presented temperatures under 20°C, 15% showed temperatures over 23°C. More than two-thirds (71%) of the classrooms had a relative humidity between 30% and 60%. The obtained correlation coefficients between measured temperature, relative humidity and VOCs, aldehydes, and particulate matter are shown in Table 2. There were significant correlations between relative humidity and toluene, m/p-xylene, d-limonene,

Table 2 Spearman rank correlation coefficients for VOCs, aldehydes, particulate matter, bioaerosols, CO_2, CO, temperature, and relative humidity levels

	Benzene	Toluene	m/p-xylene	o-xylene	d-limonene	α-pinene	Styrene	Total VOC	Formaldehyde	Acetaldehyde	CO_2	$PM_{2.5}$	PM_{10}	Temperature	Relative humidity	Bacteria	Fungi
Benzene	1																
Toluene	0.193	1															
m/p-xylene	0.080	0.824**	1														
o-xylene	−0.072	0.652**	0.801**	1													
d-limonene	0.295*	0.086	0.122	0.029	1												
α-pinene	0.218	0.526**	0.452**	0.441**	0.393**	1											
Styrene	0.103	0.172	0.175	0.171	0.204	0.029	1										
Total VOC	0.149	0.442**	0.441**	0.408**	0.451**	0.482**	0.026	1									
Formaldehyde	−0.187	0.366**	0.255*	0.426**	−0.232*	0.285*	−0.006	−0.055	1								
Acetaldehyde	0.084	0.356**	0.357**	0.334**	0.160	0.401**	0.044	0.160	0.476**	1							
CO_2	0.193	0.420**	0.470**	0.286*	0.457**	0.256*	0.033	0.424**	0.097	0.418**	1						
$PM_{2.5}$	−0.128	−0.058	0.051	0.001	−0.106	0.025	0.159	−0.140	−0.065	0.077	−0.134	1					
PM_{10}	−0.163	−0.004	0.093	0.080	−0.112	0.094	0.102	−0.021	−0.034	0.024	−0.148	0.916**	1				
Temperature	−0293*	−0.068	−0.082	0.038	−0.551**	−0.102	−0.242*	−0.190	0.301*	−0.123	−0.530**	0.123	0.172	1			
Relative humidity	0.003	0.328**	0.308**	0.190	0.313**	0.311**	0.145	0.139	0.248*	0.311**	0.624**	−0.274*	−0.335**	−0.528**	1		
Bacteria	−0.210	−0.148	−0.094	−0.066	0.141	−0.184	−0.094	−0.225	0.076	0.173	0.257*	−0.031	−0.098	−0.171	0.179	1	
Fungi	0.152	0.039	−0.055	0.146	−0.386**	0.073	−0.053	−0.305**	0.459**	0.071	−0.167	0.058	0.037	0.142	0.042	0.087	1

*Correlation is significant at the 0.05 level.

**Correlation is significant at the 0.01 level.

α-pinene, formaldehyde, and acetaldehyde. Moreover, temperature and formaldehyde levels were significantly correlated ($\rho = 0.301$; $P < 0.05$). In contrast, temperature was negatively and significantly correlated with benzene, d-limonene, and styrene, i.e. the lower the temperature, the higher the concentrations of the mentioned parameters.

Estimated ventilation rates are listed in Table 1. Ventilation rates were lower than desired value of 4 l/s.occupant in almost all classrooms (Wargocki, 2013). However, the values correspond to measurements made during unoccupied conditions, when windows and doors were closed to a greater extent than when the classroom was occupied.

Associations between schools/classrooms characteristics and occupant behavior with IAQ parameters

The Scree Plot suggested the existence of three components (Table S3). The first component explains 19% of variance and was characterized by these variables: total VOCs, toluene, m/p-xylene, and o-xylene; the second factor has 16% of variance explained and CO_2, relative humidity, $PM_{2.5}$, PM_{10}, trichloroethylene and bacteria characterized this component. Finally, the third factor explains 10% of variance and was characterized by six IAQ parameters: CO_2, CO, temperature, benzene, styrene, and d-limonene. Three of these indicators (total VOCs, PM_{10}, and CO_2) were selected for multilevel analysis.

The results of multilevel analysis are presented by the estimated linear regression coefficients of the classroom and school features and the respective 95% CI as well as intra-class correlation coefficient (ICC). Ceiling height, window area and the number of windows usually open in the cooling season were the characteristics that showed an effect on classroom CO_2 levels, explaining 16% of the differences among schools buildings (Table S4). None of the school characteristics represent a significant effect for this parameter.

Concerning the PM_{10}, the characteristics that showed a significant effect in the classroom, explaining 28% of the differences among schools were these: the number of windows usually open in the heating season; visible damp spots on walls, ceilings or floors; main ceiling surface material; visible mould growth in the room; and, the presence of a closet or shelves with gouaches, inks, etc., for graphic arts. Gasoline dispensing facilities nearby, car park sources of outdoor air pollution and the existence of a laboratory were the characteristics that contributed to the school levels of PM_{10}, explaining 33% of differences between schools (Table S5).

When the total VOC parameter was analysed, it was found that the characteristics of the school building had no significant effect on it. The number of windows usually open in the cooling season, the main floor surface material, the number of windows usually open before classes, and the presence of a closet or shelves with gouaches, inks, etc., for graphic arts are the variables that explain 21% of the differences among the schools evaluated (Table S6).

To summarize the effect of all variables resulting from each of the parameters analysed individually, a multilevel regression analysis was performed using the same model for each of the parameters studied. There was an increase in the value of the ICC, for the parameter CO_2 to 20% and parameter PM_{10}, reaching the ICC of 40%. On the other hand, a decrease was perceived, assuming a value of 16% of this coefficient, when the total VOC parameter was analysed (Table 3).

Within and between-school variation in IAQ parameters

For the dependencies of measurements taken in a given school building, within- and between-school variability was evaluated. Mixed linear models showed that concentrations of m/p-xylene varied more within schools (expressed as a percentage of the total variation) than between schools. The remaining IAQ parameters showed the opposite trend. A major variation within and between schools was observed for m/p-xylene and the lowest for temperature and relative humidity.

Table 3 Estimated linear regression coefficients of the classroom/school features and respective 95% confidence intervals for the three parameters of indoor air quality, assuming a multilevel model with 'school' as a random effect

	$\ln(CO_2)$ Estimates (95% CI)	$\ln(PM_{10})$ Estimates (95% CI)	ln(total VOC) Estimates (95% CI)
Ceiling height (m)	0.657 (0.15; 1.16)	−0.290 (−0.84; 0.26)	0.057 (−0.94; 1.05)
Windows area (m^2)	−0.024 (−0.05; 0.01)	0.003 (−0.03; 0.03)	−0.006 (−0.06; 0.04)
No. of windows usually open in the cooling season	0.067 (−0.02; 0.16)	0.045 (−0.05; 0.14)	−0.113 (−0.29; 0.06)
No. of windows usually open in the heating season	−0.123 (−0.28; 0.03)	0.127 (−0.02; 0.28)	−0.227 (−0.53; 0.08)
Visible damp spots on walls, ceiling or floor	−0.012 (−0.26; 0.24)	0.371 (0.10; 0.64)	0.340 (−0.15; 0.83)
Main ceiling surface material	−0.025 (−0.38; 0.33)	−0.312 (−0.70; 0.08)	−0.009 (−0.72; 0.70)
Visible mould growth in room	0.066 (−0.18; 0.31)	−0.245 (−0.50; 0.01)	0.046 (−0.43; 0.52)
Existence of a closet or shelves with gouache, inks etc. for graphic arts	0.082 (−0.22; 0.39)	−0.106 (−0.46; 0.25)	0.555 (−0.05; 1.16)
Windows usually open before classes	−0.034 (−0.36; 0.29)	0.160 (−0.20; 0.52)	−0.661 (−1.30; −0.02)
Main floor surface material	0.035 (−0.28; 0.35)	−0.001 (−0.37; 0.37)	0.557 (−0.05; 1.17)
Variance of school (%)	1.88	4.24	6.02
ICC (%)	20	40	16

ICC, Intra-class correlation coefficient.

Table 4 Within and between-school variation in IAQ parameters

		Percent of variation (%)		
Parameters	*n*	Within schools	Between schools	*P*-value
Benzene	6	104	254	<0.05
Toluene	20	37	227	<0.05
m/p-xylene	20	746	338	<0.05
o-xylene	20	34	191	<0.05
d-limonene	20	48	124	<0.05
α-pinene	20	44	172	0.002
Styrene	20	78	96	<0.05
Total VOC	20	38	88	<0.05
Formaldehyde	20	24	70	0.002
Acetaldehyde	20	24	84	0.001
$PM_{2.5}$	20	24	45	<0.05
PM_{10}	20	23	39	<0.05
Bacteria	20	50	65	<0.05
Fungi	20	40	74	<0.05
CO_2	20	22	35	0.001
Temperature	20	5	10	<0.05
Relative humidity	20	7	19	<0.05
Ventilation rate	20	70	156	<0.05

Between-school difference of all IAQ parameters were statistically significant at $P < 0.05$.

Within-school variability in bioaerosols, CO_2 concentrations, and ventilation rates were lower than between-school variability (Table 4).

Discussion

Concentrations and sources of IAQ parameters

According to Kotzias et al. (2005), toluene has been used as a solvent in a variety of household products such as paints, cleaning agents, adhesives, and printing products. Toluene levels measured in this study were far below the weekly average concentrations found in libraries, offices, newspaper stands, and copy centers in Italy (Bruno et al., 2008), but were similar to those reported by Stranger et al. (2007) in primary schools in Belgium, and by Martins et al. (2012) in Portuguese schools. Xylenes are widely used in the chemical industry as solvents for products such as paints, inks, dyes, adhesives, and detergents (Sarigiannis et al., 2011). In the current study, indoor xylene concentrations were higher than those registered in previous studies involving 14 elementary schools in Lisboa (Pegas et al., 2011a). Terpenes, including d-limonene, are well-known substances emitted from cleaning products and room fresheners (Singer et al., 2006). Although in the current study the high proportion of d-limonene indoors agrees with other observations made of the increasing ubiquity of this compound in indoor environments (Weschler, 2004), the entire d-limonene concentration range was much lower than the recommended limit value proposed by EU-INDEX project (450 $\mu g/m^3$) (Kotzias et al., 2005). The presence of d-limonene was identified in both indoor and outdoor air samples but with higher concentrations in the indoor environment ($I/O > 6$) suggesting the importance of indoor sources for this compound.

Total VOCs levels measured in this study were higher than in previous studies (Godwin and Batterman, 2007; Smedje et al., 1997; Zhang et al., 2006), but lower than those measured by Yang et al. (2009). Comparisons of total VOC levels across studies can be problematic due to differences in definition, sampling times, measurement, and analysis (Zhang et al., 2006), and examination of specific VOC species is often more informative. The current study showed an increase in total VOC levels when the floor surface material was PVC/vinyl or linoleum. Decreases in total VOC levels were associated with the increase of ventilation measured by the number of windows usually open in the cooling season and if the windows were usually open before classes. These results, the room-to-room variability, and the outdoor levels suggest classroom (indoor) sources rather than building-wide or outdoor sources.

Indoor concentrations of formaldehyde and acetaldehyde exceeding the outdoor concentrations suggest that indoor sources were the most important contributors to the indoor levels. Indoor formaldehyde concentrations may be related to insulating materials; parquet, particle board or plywood furniture containing formaldehyde-based resins; and paints, cleaning and other consumer products used either in the didactic work or in the cleaning processes of the classrooms (Gilbert et al., 2008; Mendell, 2007). Additionally, formaldehyde and acetaldehyde can occur in the indoor environment as secondary product, and therefore, as products of the reaction of a primarily emitted pollutant with ozone (Nazaroff and Weschler, 2004). The formaldehyde concentrations measured in the present study were higher than those reported for schools in Sweden (Smedje et al., 1997) and Australia (Zhang et al., 2006), but lower than the median concentration reported in France (Annesi-Maesano et al. (2012). Taking into consideration that each classroom was equipped with standard plywood school furniture, and that currently no special care was taken regarding the household products used in the classrooms, particular attention should be paid regarding the selection of new furniture, cleaning, consumer, and didactic products.

There was a significant correlation between relative humidity and almost of the measured VOCs and aldehydes. Therefore, an increase in classroom relative humidity may result in high levels of the mentioned chemical agents.

$PM_{2.5}$ concentrations have been measured in few studies (Annesi-Maesano et al., 2013). As the classrooms do not contain any specific $PM_{2.5}$ source (such as smoking or cooking), the indoor $PM_{2.5}$

concentrations were more likely due to outdoor pollution penetration rather than indoor sources related to the presence of children. This finding is consistent with the observations from other studies (Almeida et al., 2011; Fromme et al., 2007; Guo et al., 2010; Oeder et al., 2012). Due to the fact that 13 (65%) of the schools in the present study were situated close (<500 m) to a heavily trafficked road and that 5 (25%) were close (<100 m) to a car park, it is expected that ambient air did contribute to indoor concentrations of particulate matter in the classrooms. It could therefore be suggested that when new schools are built, outdoor risks factors should be taken into account. In general, the indoor PM_{10} concentrations obtained were consistent with data reported in other studies (Fromme et al., 2008; Simoni et al., 2010), but higher than those reported by Stranger et al. (2007). Re-suspension of coarse particles indoors resulting from occupant activities as well as the presence of other potential indoor sources of coarse particles were important factors to the increase PM_{10} concentrations indoors. Besides delayed deposition/settlement due induced turbulence created by occupant's movements and the reduced ventilation could also affect the dispersion of PM_{10}, and so causing their accumulation indoors. The PM_{10} indoor concentration profiles showed peaks within the time slots when the studied classrooms were occupied (Madureira et al., 2012). However, the data from current study should be observed with caution taking into account that indoor and outdoor particulate matter could not be sampled in parallel, affecting the accuracy of the estimated I/O ratios.

In the present study, approximately 20% of the classrooms had interior damp stains and 49% of classrooms had a tendency to form condensation on the windows. This excess of moisture could be associated with higher levels of bacteria as reported by Meklin et al. (2002). Moreover, the environmental conditions surrounding the classrooms, as plants and soil in the school playgrounds can offer important sources of microorganisms (WHO, 2009). Mean indoor levels of bacteria higher than 500 CFU/m^3 were observed in 63 (86%) classrooms; which was similar to those obtained in 11 schools in Porto during the winter season (Madureira et al., 2009), but lower than those reported in a study covering 14 schools in Lisboa in spring (Pegas et al., 2011a). Indoor bacteria concentrations were higher when compared with outdoor levels, indicating significant indoor sources and poor ventilation. For both the indoor and outdoor air samples, the concentrations of fungi were lower than the concentrations of the bacteria, which were consistent with other studies (Godwin and Batterman, 2007). The weather conditions including low temperatures and precipitation levels could explain lower outdoor concentrations. During the heating season, occupants generally spend more time in indoor environments, windows are more often closed, due to the outdoor weather conditions or due to the fact that heating systems were turned on, and ventilation may be insufficient; thus, indoor temperature and relative humidity become suitable for fungal growth indoors as reported by (Meng et al., 2012), which is in agreement with the results obtained in the current study. None were statistically significant, although a positive Spearman correlation coefficient was observed ($\rho = 0.142$, $P > 0.05$ between temperature and fungi; and $\rho = 0.042$, $P > 0.05$ between relative humidity and fungi). Madureira et al. (2014) also found a positive correlation between indoor concentration of airborne fungi and indoor temperature ($\rho = 0.453$, $P < 0.05$), which is consistent with the fact that optimal temperature ranges for fungal growth may have been achieved for some fungi genera or species. The median fungi level was higher than those values reported in other studies conducted in similar places (Grisoli et al., 2012; Mentese et al., 2009; Roda et al., 2011).

Based on CO_2 levels, inadequate ventilation appears to be a common IAQ problem encountered in the studied classrooms, reinforcing earlier studies (Geelen et al., 2008; Madureira et al., 2009; Mumovic et al., 2009; Pegas et al., 2011a). Based on a 1000 ppm CO_2 limit (ASHRAE 62-2001, 2001) and using school-day averages, 86% of the classrooms were inadequately ventilated. In addition, classrooms were monitored under 'closed' conditions, keeping windows and doors closed as best possible during the occupied hours. During the occupation period it was observed that CO_2 concentration produced by the occupants build up until reaching an equilibrium level reaching levels greater than 1000 ppm and decreased to levels below 1000 ppm during breaks (data not shown).

Multiple regression models were performed to assess the associations between the schools/classrooms characteristics, occupant behavior, and the CO_2 levels. The present study showed higher CO_2 concentrations in classrooms with higher ceiling height and an inverse association with windows area in the classroom. Although almost all classrooms have the same ceiling height (range = 2.9–3.6 m), the classroom area/volume and the density of occupation varied between classrooms. Moreover, the difficulty associated to heat a high space volume might also explain and determine the occupant behavior reflected in a reduced number of times that the windows were opened (introduction of 'fresh' air); thus, suggesting a potential stagnation of the indoor air. Consequently, taking into account that the school staff reported that opening windows was not so frequent due to noise problems and/or weather conditions, the results of the present study underlined the relevance of use strategies or occupant behavior influencing indoor CO_2 concentrations.

Two parameters are particularly critical: the density of occupation and the duration time of both 'teaching periods' and 'breaks'. The implementation of more breaks and recesses between classes, and decreasing the occupancy per classroom might help to reduce the indoor levels of pollutants that originate from indoor sources.

Spatial variation

School-by-school variation for most IAQ parameters, except for temperature and relative humidity, suggests that differences in outdoor location and building-wide cleaning/maintenance practices affected measurements more than any common classroom factor. Consequently, multiple building locations should be measured to characterize IAQ parameters in schools.

Strengths and weaknesses of the study

The current study had a large sample size in particular when compared with earlier studies carried out in Portugal (Madureira et al., 2009; Martins et al., 2012; Pegas et al., 2011a; Sousa, 2009; Valente, 2010). In addition, measurements were performed using standardized procedures and the objective measurement of a broad spectrum of IAQ parameters in classrooms allowed a better appraisal of individual exposure compared to indirect methods such as the use of questionnaires or checklists (Viegi et al., 2004). However, results may not be representative of school districts elsewhere for several reasons (e.g. climatic zones and ambient air quality, as well as building characteristics). Moreover, schools were monitored during the winter. Monitoring during other seasons is necessary to evaluate seasonal effects, e.g. ventilation may be further increased during warm seasons. Furthermore, the IAQ characterization used instruments, indicators, averaging times and analysis methods that may differ from those used in other studies, especially for VOCs, particulate matter and bioaerosol measurements. For the specific case of VOCs and aldehydes, the passive sampling method provided an integrated sample for both occupied and unoccupied periods; thus concentrations may not be representative of occupant exposure levels. Depending on the nature of the source and the ventilation, passive sampling may either over- or underestimates occupant exposures, especially for VOCs closely associated with occupant activities. Another limitation can be associated with the estimation of ventilation rate based on the decay of CO_2 concentrations measured in the classrooms after the school was closed and the classroom was empty with all windows and doors closed to a greater extent at the times when the classroom was occupied. Future studies utilizing other methods for ventilation rates estimation (Godwin and Batterman, 2007; Haverinen-Shaughnessy et al., 2011; Mendell et al., 2013) would be used for comparison purposes. However, a basic challenge is that a CO_2 approach utilized for the estimation of ventilation rates (with children in classroom) will be affected by levels of activity that will typically vary throughout a given school day. Moreover, as reported by (Haverinen-Shaughnessy et al., 2011) classrooms environments are difficult to characterize because of the activities that typically are non-stop in children with 8–10 years old as occurred in the current study. In addition, monitoring did not include other potentially important contaminants (e.g. ultrafine particles, endotoxins, allergens). Bioaerosol sampling over short periods in microenvironments and the used of culture-based sampling could be problematic. To overcome it, measurements were systematically obtained during periods of typical activities in the classrooms after an extended period of steady occupancy. However, for future sampling we would also recommend greater use of repeated measurements over the school week and a larger sample size of schools.

Conclusions

The 73 classrooms monitored in 20 public primary schools located in Porto showed generally low levels of VOCs and aldehydes, acceptable ranges of temperature and humidity, but often high levels of CO_2, $PM_{2.5}$, PM_{10}, bacterial concentrations, and low rates of ventilation, which might be explained by the reduced airing of the classrooms which underlines the influence of indoor sources, occupant behavior and maintenance/cleaning activities in schools and the high density of occupants.

The between-school variability of most IAQ parameters (most VOCs, bioaerosols, and CO_2) exceeded the variability within schools, suggesting the influence of activities or building features and the need for multiple monitoring locations to characterize IAQ in schools. For VOCs, identified sources included graphic art activities and floor surface material (PVC/vinyl, linoleum).

Therefore, we recommend that school buildings be designated to prevent indoor pollutant sources. That situation could be overcome by the implementation of more breaks and recesses between classes, decreasing the occupancy per room, increasing the exchange of indoor air with the outdoor, and improving the cleanliness of facilities which might benefit the IAQ. The advice is to adopt strategies based on source control as the most consistent and efficient for the prevention of adverse health consequences to children and adults in schools. Nevertheless, to completely explore how building and classroom characteristics may influence the IAQ in schools, a study with a larger sample size needs to be conducted.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Characteristics of the classrooms (n = 73).

Table S2. Indoor/outdoor ratio for VOC, aldehydes, particulate matter, bioaerosols, CO_2, CO, temperature and relative humidity levels.

Table S3. Rotated component matrix with *varimax* rotation obtained of the principal components analysis method.

Table S4. Estimated linear regression coefficients of the classroom features and respective 95% confidence intervals for the parameter CO_2, assuming a multilevel model with 'school' as a random effect.

Table S5. Estimated linear regression coefficients of the classroom/school features and respective 95% confidence intervals for the parameter PM_{10}, assuming a multilevel model with 'school' as a random effect.

Table S6. Estimated linear regression coefficients of the classroom features and respective 95% confidence intervals for the parameter total VOC, assuming a multilevel model with 'school' as a random effect.

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