Indoor air quality in schools and its relationship with children's respiratory symptoms

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HIGHLIGHTS

- Indoor air measurement campaigns in 73 classrooms from 20 public primary schools.
- Children health information obtained using a questionnaire and clinical tests.
- Relationships between IAQ and children's respiratory symptoms.
- Even at low levels indoor air pollutants were related with the respiratory symptoms.

ARTICLE INFO

ABSTRACT

A cross-sectional survey was conducted to characterize the indoor air quality (IAQ) in schools and its relationship with children's respiratory symptoms. Concentrations of volatile organic compounds (VOC), aldehydes, $PM_{2.5}$, PM_{10}, carbon dioxide, bacteria and fungi were assessed in 73 classrooms from 20 public primary schools located in Porto, Portugal. Children who attended the selected classrooms ($n = 1134$) were evaluated by a standardised health questionnaire completed by the legal guardians; spirometry and exhaled nitric oxide tests.

The results indicated that no classrooms presented individual VOC pollutant concentrations higher than the WHO IAQ guidelines or by INDEX recommendations; while $PM_{2.5}$, PM_{10} and bacteria levels exceeded the WHO air quality guidelines or national limit values. High levels of total VOC, acetaldehyde, $PM_{2.5}$ and PM_{10} were associated with higher odds of wheezing in children. Thus, indoor air pollutants, some even at low exposure levels, were related with the development of respiratory symptoms. The results pointed out that it is crucial to take into account the unique characteristics of the public primary schools, to develop appropriate control strategies in order to reduce the exposure to indoor air pollutants and, therefore, to minimize the adverse health effects.

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1. Introduction

Because of their immature immune and respiratory system, inferior body mass index and breathing pattern children are more susceptible to the effects of air pollution than adults. Asthma and allergy are two of the most prevalent diseases in children (Pearce et al., 2000). Moreover, both diseases are often associated being asthma the culminant disease resultant from the atopic march (Bantz et al., 2014). There is evidence of the increased prevalence of asthma and allergies over the recent decades, especially in developed countries, among children (WHO, 2007; Lotvall et al., 2009). It has been reported that more than a third of children in Europe has had bronchial asthma or allergy (Asher et al., 1998).

The aforementioned increase is assumed to be multi-factorial and to result from complex interactions between genetic predisposition and environmental factors. Among the latter, indoor air

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pollution has assumed a particular major relevance (Masoli et al., 2004). Indoor air has been the focus of scientists during the last decade, specifically due to the fact that people spend most of their time indoors, especially at home and in school.

Indoor air quality (IAQ) is determined by a combination of numerous pollutants originated from a wide spectrum of pollution sources, with all of them having specificities associated to the place, the climate and the culture: the local ambient air, the buildings characteristics and the indoor activities (Oliveira Fernandes et al., 2008). The IAQ in schools is generically characterized by a complexity of various pollutants namely volatile organic compounds (VOC), particulate matter, aldehydes, bacteria and molds (Madureira et al., 2009, 2012).

Indoor air pollutants can cause or contribute to short-term and long-term health problems (Clausen et al., 2009; Simoni et al., 2010; Annesi-Maesano et al., 2013). Moreover, indoor air pollutants can provoke discomfort and reduce school attendance and productivity (Mendell and Heath, 2005). Despite the large population of primary schoolchildren, only a few studies regarding IAQ in Portuguese primary schools have been undertaken (Madureira et al., 2009; Martins et al., 2012; Pegas, 2012). Often a specific pollutant, e.g. particulate matter or bioaerosols, or a combination of pollutants, are addressed, and just a few studies, have used objective measurements of IAQ and health indicators, such as spirometry and exhaled nitric oxide (eNO).

In order to improve the indoor environmental conditions in schools, which represent one of the major contributor of children's total exposure (Bluyssen, 2014), and, thus, to limit exposures that may cause or contribute to asthma, allergy and other respiratory symptoms in children, the main objective of this work was to conduct a comprehensive characterization of the IAQ in schools and its relationship with children's respiratory symptoms.

These findings are of relevance to public health due to the very large population of exposed school children since the attendance in primary schools is compulsory and asthma and allergy are very common diseases in childhood. These data may be useful for assessing the health effects of exposure, for understanding the underlying mechanisms and for implementing preventive policies in terms of standards and guidelines.

2. Material and methods

Due to budget and time limitations, a cross-sectional survey was carried out in 20 public primary schools located in Porto, North of Portugal at the sea shore (41°N, 8W) featuring a Mediterranean climate with moderate temperatures and rainy weather in the winter season (Fig. 1S, in the Supplementary Material).

The number of schools was defined based on the estimated sample size of children to study the relation between IAQ and asthma, allergy and respiratory symptoms. In regard to the figures obtained in recent studies in Portugal, the prevalence of asthma in children is approximately 10% while it is estimated that 10% of non-asthmatic children have symptoms and that the exposure to poor indoor air leads to a two times higher risk of having symptoms (Falcão et al., 2008). Within these premises, a sample of 1600 children was established. Thus, assuming 20 children per room (Direção-Geral de Estatísticas da Educação e Ciência, 2014) and 4 classrooms per school, a sample size of 20 schools was used to give sufficient confidence. Thus, the 20 (38%) schools with the highest number of students were selected from a total of 53 public primary schools (Table 1S).

Depending on the size of the school, two to four classrooms per school, comprised of 8—10 year old children, 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. No classrooms had mechanical ventilation systems; opening windows was the only way to renovate the air indoors. In the winter season, the windows were closed due to the outdoor weather conditions and/or due to the fact that heating systems were turned on.

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.

2.1. Indoor air quality assessment

School visits and air sampling were conducted in the winter seasons, from November to March, during years 2011—2013. Measurements of VOC, aldehydes, $PM_{2.5}$, PM_{10}, bacteria, fungi, temperature and relative humidity levels were conducted simultaneously both indoors and outdoors during a 5-day period (school week, from Monday morning until Friday afternoon). The air samplings were performed during regular daily activities, except for VOC and aldehydes, and under representative conditions of occupancy and use of the classrooms.

2.2. Air sampling and analysis

Safe and childproof sampling locations were selected complying ISO 16000-1 (2004). Indoor samples were collected near occupants' breathing zone (approximately 0.7—1.5 m above the floor). Sampling locations were no closer than 1 m to a wall, window, door, or an active heating system. Furthermore, the indoor sampling locations were selected as far away as possible from the blackboard, when applicable. The sampling process was supervised by a researcher who recorded information regarding classroom occupancy, ventilation and occupant behaviour and activities. Outdoor samples were obtained concurrently to indoor air sampling whenever possible no closer than 1 m from the building at heights of 1—2 m above the ground.

Volatile organic compounds were collected using stainless-steel sampling tubes containing Tenax® TA (60/80 mesh). Tenax tubes were transferred to the laboratory and 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), according to 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. The indoor and outdoor air sampling covered a 5-day period (school week, from Monday morning until Friday afternoon). To control contamination during transport and sampling, a field blank was employed in every school. All samples were taken in duplicate to verify the reproducibility of measurements. 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) 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 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/m³ for

formaldehyde and 0.178 $\mu g/m^3$ for acetaldehyde.

Portable TSI DustTrak DRX photometers (model 8533; TSI Inc.) were used for the assessment of $PM_{2.5}$ and PM_{10} concentrations. This equipment measures particles with a laser photometer based on light scattering principle. The measuring range of the equipment is $1-150 \times 10^3$ $\mu g/m^3$ with accuracy of $\pm 0.1\%$ of reading of 1 $\mu g/m^3$, and it 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 (8 h, avoiding Mondays and Fridays). Logging intervals were set to 1 min between each sample. Similarly to the indoor sampling, another TSI DustTrak DRX photometer was also used for the assessment of outdoor $PM_{2.5}$ and PM_{10} concentrations. Instruments were installed outside in the school playground, at a high of $1-1.2$ m to simulate the children's breathing zone, protected from rain and carefully leaving the inlet uncovered to not disturb the sampling. The instrument was set to continuously measure the outdoor $PM_{2.5}$ and PM_{10} during at least one school day (8 h, avoiding Mondays and Fridays). Logging intervals were also set to 1 min between each sample. The monitors are zeroed automatically using the external zeroing module. By zeroing the monitors during sampling, the effect of zero drift is minimized. Meanwhile, the two available 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 EN 13098 (2000). Tryptic Soy Agar (TSA) (supplemented with 0.25% cicloheximide) and Malt Extract Agar (MEA) (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 (duplicates of 100 and 250 L) were collected both indoors and outdoors between 9.30 a.m. and 12.00 a.m. 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 sealed, marked and 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 (European Standards, 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 number of colonies recovered on the air-sample plates was adjusted using a positive-hole correction factor, and the results were expressed as number of colony forming units per cubic meter of air (CFU/m^3). The correction factor was based on the Fellers law (Andersen, 1958). The quantification limit is established as 10 CFU per plate.

Carbon dioxide (CO_2), temperature and relative humidity levels were recorded concurrently with the other air parameters (both indoors and outdoors) using an IAQ-CALC monitor (model 7545, TSI, Inc.). The equipment combined an infrared non-dispersive sensor for CO_2, an thermistor for measuring temperature in a range from 0 to 60 °C with an accuracy of ± 0.6 °C, and a thin-film capacitive sensor for relative humidity (range of $5-95\%$ relative humidity; accuracy $\pm 3.0\%$ relative humidity). Measurements were conducted during one school week with a time step of 5 min during the investigation week both indoors and outdoors.

2.3. Health-related data collection

The child health data was obtained using a questionnaire completed at home by legal guardians, based on the ISAAC (International Study of Asthma and Allergies in Childhood) questionnaire; and by a clinical examination including spirometry and eNO tests performed at school by trained health professionals. Lung function measurement by spirometry permits the discrimination of participants with respiratory symptoms and is invaluable as a screening test for general respiratory health (Mortimer et al., 2003; Miller et al., 2005). Exhaled nitric oxide is considered as a readout of certain aspects of airway inflammation, particularly in atopic asthma. Exhaled nitric oxide was used as a non-invasive marker of asthmatic inflammation and has several characteristics (instantaneous, non-invasive, repeatable, safe) that make it ideally suited for children (Prieto, 2002; Hatziagorou and Tsanakas, 2007).

2.3.1. Questionnaire

The questionnaire was filled in by legal guardians and comprised questions about respiratory/allergic health of the child; current symptoms/diagnosis; environmental tobacco smoke exposure, perinatal information, dietary habits, socio-economic characteristics and building characteristics of the home, in particular the child's bedroom (e.g. outdoor traffic pollution, dampness, floor and wall coverings, cooking fuel types; air conditioning).

The health outcomes analysed in the current study are based on the following questions: *(i)* wheeze (<12 months): "In the past 12 months, has your child had wheezing or whistling in the chest?"; *(ii)* wheeze (<30 days): "In the past 30 days, has your child had wheezing or whistling in the chest?"; *(iii)* ever nasal allergy: "Has your child ever had nasal allergies, including hay fever?"; *(iv)* cough episodes: "Does your child have cough on most days (four or more days per week) apart from common colds?"; and *(iv)* phlegm episodes: "Does your child have phlegm on most days (four or more days per week) apart from common colds?".

2.3.2. Clinical examination

The clinical tests were performed at school during a normal school period following standardised procedures, in one day of the week of the IAQ measurements in the classrooms. All participant children were invited to measure weight, height and lung function. Exhaled nitric oxide was measured in a sub-sample of five children from each classroom. These children were randomly selected from the total participants.

Body weight was measured using a digital scale-Tanita (Tanita® TBF-300, Tanita Corporation of America, Inc., Illinois, USA) (in kilograms, to the nearest tenth), and height was measured (in centimetres, to the nearest tenth) using a portable stadiometer (SECA® 214). Both were measured with the child upright and standing without shoes. Then, the body mass index (BMI) was calculated [weight (kg)/m^2].

Lung function was measured using a spirometer (ML3500, MicroLab®). All spirometry tests were performed according to American Thoracic Society/European Respiratory Society guidelines (1995) and the indexes measured or derived from lung function were referenced to predicted values (%): forced vital capacity (FVC), forced expiratory volume in 1 s (FEV_1), FEV_1/FVC ratio and forced expiratory flow $25-75\%$ ($FEF_{25-75\%}$). In brief, immediately following a full inhalation, the children seals his/her lips around the mouthpiece and blasts the air out as fast as possible until the lungs are absolutely empty. Demonstration to the children of the procedure and the maximal effort required was performed before starting, and the best of three technically acceptable tests was considered the final result.

The levels of eNO were measured according to the ATS/ERS guidelines (2005) with the handheld device NIOX MINO system (Aerocrine, Stockholm, Sweden) with a detection limit of 5 ppb. The analyser provides online continuous measurement of nitric oxide. After children exhaled residual volume, they inserted the mouthpiece, inhaled nitric oxide free air from the apparatus to the total

lung capacity and then exhaled for 10 s at a constant flow rate of 50 ml/s. The end point of measurement occurred when a plateau for at least 2 s was observed. A visual feedback helped the children achieve the desired expiratory flow of 50 ml/s ($\pm 10\%$).

2.4. Participants

A total of 1639 children were invited to participate in the survey. One hundred and eighty one (11.0%) parents refused and 324 (19.8%) did not return the signed consent form and parent questionnaire and were, therefore, considered as refusals (participation rate of 69.2%). Out of 1099 children (mean age 8.6 $\pm$ 0.7 years) who participated in parents questionnaire, for the detailed analyses, 121 children were excluded due to with missing information on at least one of the following outcomes (ISAAC questions): wheeze (<12 months), wheeze (<30 days), ever nasal allergy, cough episodes and phlegm episodes. The final sample was 978 children (508 girls) to study respiratory symptoms; 761 (392 girls) concerning lung function and 318 (165 girls) regarding the eNO test.

2.5. Statistical analysis

Shapiro–Wilk test was used for normality testing. The distribution of all indoor air parameters were skewed; thus they were described by median, 25th percentile (P25) and 75th percentile (P75). $PM_{2.5}$, PM_{10}, temperature and relative humidity levels were calculated from the data collected during teaching periods; while data of the VOC and aldehydes are reported for the school week under observation. These parameters were categorized into three classes according to the tertiles of exposure. Concentrations of VOC below the detection limits were excluded from the statistical analyses. It is noteworthy that in order to correctly explore the relationship between indoor and outdoor, the data used for the I/O ratios analysis were those collected during the same period from indoor data. Kruskal–Wallis test was used to compare continuous variables.

To evaluate the association between indoor air exposure and respiratory symptoms, odds ratio (OR) and respective 95% confidence intervals (95% CI), were computed, using logistic regression models. Adjustments have been made for age (continuous variable in years), sex, mother's education level (categorical variable measured as the number of successfully completed years of formal schooling: 0–6 years; 7–9 years; 10–12 years and ≥ 13 years), BMI (continuous variable), relative humidity and temperature (both as continuous variable).

Statistical analyses were performed using SPSS Statistics version 19. A p-value below 0.05 was considered statistically significant.

3. Results

The summary of the indoor measurements taken in the 73 classrooms is presented in Table 1. Tables 2S and 3S, in the Supplementary Material, provides the outdoor measurements and indoor/outdoor ratio for air parameters, respectively.

The median TVOC concentration was 140.3 μg/m³ (P25–P75 = 85.5–198.4 μg/m³). Among the indoor VOC concentrations, D-limonene presented the highest levels (23.1 μg/m³) followed by toluene (6.37 μg/m³); while benzene, m/p-xylene, o-xylene, α-pinene, trichloroethylene, tetrachloroethylene, naphthalene and styrene had median levels lower than 5 μg/m³. Indoor levels of individual VOC were higher than outdoor levels being only significant for D-limonene (23.1 μg/m³ vs. 2.05 μg/m³, $p = 0.001$) (Table 2S) and as expected, indoor concentrations usually exceeded outdoor levels; being significant only for D-limonene ($p = 0.001$) (Table 3S). The high indoor/outdoor ratios (I/O > 6) for D-limonene,

formaldehyde and acetaldehyde, and the moderate I/O ratio (~2) for TVOC and toluene suggest that indoor sources are the main origin for these VOC. In contrast, the I/O ratio for benzene (0.84) indicates that outdoor sources were the primary contributors (Table 3S). No classrooms presented individual VOC pollutant concentrations higher than the WHO IAQ guidelines (WHO, 2010) or the INDEX recommendations (Kotzias et al., 2005). Classrooms with graphic art activities (e.g. painting) had some of the highest levels measured for certain VOC (e.g., toluene and naphthalene, respectively).

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); being significantly higher than those measured outdoors (17.5 vs. 2.74 μg/m³, $p < 0.05$). The indoor median concentration of $PM_{2.5}$ and PM_{10} in all classrooms exceed the 25 μg/m³ and 50 μg/m³ guideline values suggested by WHO air quality guidelines for a sampling period of 24 h (WHO, 2005). Outdoor levels of $PM_{2.5}$ and PM_{10} ranged from 27 to 270 μg/m³ and from 30 to 276 μg/m³, respectively (Table 2S). Whilst for $PM_{2.5}$ there was no significant difference with levels measured outdoors and in classrooms (71 vs. 82 μg/m³, $p = 0.098$); for median PM_{10} indoor concentration was significantly higher than outdoor (75 vs. 127 μg/m³, $p = 0.001$) (Table 2S). The indoor concentrations exceeded outdoor levels, leading to an I/O ratio higher than the unity, which suggests possible indoor sources (Table 3S).

The classrooms had a median concentration of bacteria higher than 1000 CFU/m³. In 35 (48%) classrooms, indoor levels were higher than 3000 CFU/m³ (Table 1). There was a significant difference between indoor and outdoor levels of bacteria, being higher indoors (3224 CFU/m³ vs. 213 CFU/m³, $p < 0.01$) (Table 2S), with an I/O ratio higher than the unit (Table 3S). Indoor fungi concentrations ranged from 61 to 1322 CFU/m³ (median = 240 CFU/m³). Although there were no significant differences between indoor and outdoor levels of fungi (240 CFU/m³ vs. 200 CFU/m³, $p = 0.066$) (Table 2S) in 43% of classrooms the fungi levels were above the Portuguese legislation ("concentration of fungi indoors < concentration of fungi outdoors") (Ordinance 353-A/2013 of 4th December).

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. 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 3S). Higher values were measured in classrooms with higher occupancy density for the longest teaching periods between breaks.

Table 2 and Table 3 shows data regarding the association of the indoor air parameters and wheeze, ever nasal allergy, cough episodes and phlegm episodes. After adjustment, higher values of toluene and o-xylene were associated with the occurrence of wheeze; no significant association was found with other VOC, but the increase concentration of TVOC showed increased odds of wheeze in the previous month. Acetaldehyde also showed a positive association with the occurrence of wheeze, but only concerning the previous year. In addition, higher exposure levels to $PM_{2.5}$ and PM_{10} increase the odds of wheeze among children; being the association strong for PM_{10}. Higher levels of bacteria showed increased odds of cough episodes but decreased odds of wheeze;

Table 1
Summary statistics of indoor air parameters in classrooms ($n = 73$ classrooms).

	Median	25th percentile	75th percentile	Minimum	Maximum
Benzene, $\mu g/m^3$	2.5	1.6	2.6	1.5	2.7
Toluene, $\mu g/m^3$	6.4	4.5	10.4	1.8	202.5
m/p-xylene, $\mu g/m^3$	5.0	3.3	6.8	1.2	365.2
o-xylene, $\mu g/m^3$	2.3	1.8	3.8	1.1	52.4
D-limonene, $\mu g/m^3$	23.1	11.5	48.6	2.8	215.3
α-pinene, $\mu g/m^3$	1.8	1.3	2.8	1.0	32.0
T3CE[a], $\mu g/m^3$	–	–	–	–	–
T4CE[b], $\mu g/m^3$	2.9	1.8	3.4	1.1	8.3
Naphthalene, $\mu g/m^3$	1.3	1.2	1.6	1.2	1.7
Styrene, $\mu g/m^3$	1.2	1.2	1.4	1.0	2.7
TVOC[c], $\mu g/m^3$	140.3	85.5	198.4	8.88	820.2
Formaldehyde, $\mu g/m^3$	17.5	13.8	23.1	8.24	126.9
Acetaldehyde, $\mu g/m^3$	7.7	5.0	10.4	1.9	64.6
$PM_{2.5}$, $\mu g/m^3$	82	67	106	39	244
PM_{10}, $\mu g/m^3$	127	109	167	56	320
Bacteria, CFU/m^3	3224	1784	5430	168	8372
Fungi, CFU/m^3	240	169	400	61	1322
CO_2, ppm	1469	1195	2104	829	3111
Temperature, ºC	20.8	19.2	21.7	14.3	24.6
Relative humidity, %	54	50	65	34	74

Limits of detection: 1.0 $\mu g/m^3$ for benzene; 1.1 $\mu g/m^3$ for toluene; 1.0 $\mu g/m^3$ for m/p-xylene; 1.0 $\mu g/m^3$ for o-xylene; 2.0 $\mu g/m^3$ for D-limonene; 1.0 $\mu g/m^3$ for α-pinene; 1.0 $\mu g/m^3$ for T3CE and T4CE; 1.0 $\mu g/m^3$ for naphthalene and for styrene.

[a] Trichloroethylene.
[b] Tetrachloroethylene.
[c] Total Volatile Organic Compounds.

while higher concentrations of fungi also were associated with lower odds of wheeze.

Table 4 and Table 5 presents the results of lung function tests (percentage predicted of FVC, FEV_1, FEV_1/FVC ratio and $FEF_{25-75\%}$) found in the 761 children (392 girls) that underwent pulmonary function tests according to the categories of indoor air parameters. Generally, children present normal pulmonary function and although some statistical differences were found, no relevant differences were detected according to the levels of indoor air parameters.

To investigate the association between indoor air parameters and airway inflammation, the eNO test was used. Exhaled nitric oxide was assessed in 318 children (51.9%), where 23.3% of schoolchildren had an eNO value over 20 ppb and 10.7% presented values above 35 ppb.

Since eNO could be influenced by the presence of respiratory and allergic symptoms, we stratified our sample according the presence of acute respiratory symptoms (wheeze, sneeze, runny or blocked nose). Children with symptoms or disease diagnosed by a doctor ($n = 156$) were defined based on an affirmative response to one of the following questions: "Has your child ever had wheezing or whistling in the chest at any time in the past?"; "In the past 12 months, has your child had wheezing or whistling in the chest?"; "In the past 30 days, has your child had wheezing or whistling in the chest?"; "Has your child ever had asthma diagnosed by a doctor?"; "Has your child ever had a problem with sneezing, or a runny, or blocked nose when he/she did not have a cold or the flu?"; "In the past 12 months, has your child had a problem with sneezing, or a runny, or blocked nose when he/she did not have a cold or the flu?"; "Has your child ever had nasal allergies, including hay fever?" and "If yes, was it confirmed by a physician?".

The global median (P25–P75) of eNO was 13.0 ppb (9.00–18.0 ppb), being significantly lower among children without symptoms [12.0 ppb (9.00–17.0 ppb)] than in those with symptoms [13.5 ppb (10.0–23.0 ppb)], $p = 0.021$.

Non-significant differences of eNO values were found according to indoor exposure among children without symptoms or disease diagnosed by a doctor. Among those with symptoms or disease diagnosed by a doctor increased levels of eNO were associated with

increased levels of particles and the opposite was found regarding o-xylene, bacteria and fungi concentrations. However, none of the comparisons reach statistical significance (Table 6).

4. Discussion

The 73 classrooms monitored showed low levels of VOC, but often high levels of $PM_{2.5}$, PM_{10}, and bacterial concentrations. Total VOC levels measured in this study are higher than in previous studies [Smedje et al. (1997); Zhang et al. (2006); Godwin and Batterman (2007)], but lower than those measured by Yang et al. (2009); the concentrations of individual VOC are much lower than the recommended value proposed by EU-INDEX project (Kotzias et al., 2005) and WHO (2010). The observed D-limonene concentration range is 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 additional indoor sources or inadequate renovation of air indoors as suggested by the obtained CO_2 levels (using school-day averages 86% of the classrooms had levels higher than 1000 ppm). Comparisons of VOC levels across studies can be difficult due to differences in definition, sampling times, measurement, and analysis (Zhang et al., 2006). In schools that have swimming pools, dedicated science and art classrooms, and so on, higher concentrations of certain VOC (e.g., chlorinated VOCs, aromatics) might be expected (Godwin and Batterman, 2007). According to Mendell (2007) and Zhang et al. (2006), VOC are likely originated from a combination of building sources, occupant activities, and outdoor sources. In addition to these indoor sources, the insufficient ventilation is likely to favour the increase of VOC levels.

The median values of formaldehyde levels were lower than the guidelines values established by the WHO (2010) (100 $\mu g/m^3$, 30 min sampling time) and the EU-INDEX project (30 $\mu g/m^3$, 30 min sampling time) (Kotzias et al., 2005). Indoor concentrations of formaldehyde and acetaldehyde exceeding the outdoor concentrations suggest that indoor sources are the most important contributors to the indoor levels. Indoor formaldehyde

Table 2
Associations between volatile organic compounds and wheeze, nasal allergy, cough episodes and phlegm episodes ($n = 978$).

	Wheeze (<12 months)		Wheeze (<30 days)		Ever nasal allergy		Cough episodes		Phlegm episodes	
	OR (95% CI)	aOR (95% CI)	OR (95% CI)	aOR (95% CI)	OR (95% CI)	aOR (95% CI)	OR (95% CI)	aOR (95% CI)	OR (95% CI)	aOR (95% CI)
Toluene, μg/m³										
<4.64	1	1	1	1	1	1	1	1	1	1
4.64–8.06	1.29 (0.78–2.16)	1.32 (0.74–2.35)	0.84 (0.38–1.85)	0.91 (0.38–2.14)	1.03 (0.68–1.55)	0.95 (0.60–1.51)	0.83 (0.52–1.33)	0.76 (0.44–1.31)	0.82 (0.50–1.34)	0.70 (0.40–1.21)
≥8.07	1.55 (0.94–2.57)	**1.82 (1.01–3.30)***	1.65 (0.83–3.29)	**2.44 (1.11–5.40)***	0.98 (0.65–1.49)	1.07 (0.66–1.72)	0.62 (0.38–1.04)	0.73 (0.40–1.33)	0.65 (0.39–1.10)	0.63 (0.34–1.16)
m/p-xylene, μg/m³										
<4.02	1	1	1	1	1	1	1	1	1	1
4.02–5.89	**2.03 (1.23–3.36)***	**2.35 (1.32–4.21)***	1.77 (0.83–3.77)	2.13 (0.92–4.97)	1.36 (0.91–2.03)	1.37 (0.88–2.14)	0.83 (0.52–1.35)	0.87 (0.49–1.52)	0.75 (0.46–1.23)	0.78 (0.45–1.37)
≥5.90	1.36 (0.79–2.33)	1.84 (0.96–3.52)	1.75 (0.81–3.76)	2.32 (0.94–5.73)	0.84 (0.54–1.30)	0.88 (0.52–1.46)	0.71 (0.43–1.17)	0.94 (0.51–1.74)	0.68 (0.40–1.13)	0.70 (0.38–1.30)
o-xylene, μg/m³										
<1.89	1	1	1	1	1	1	1	1	1	1
1.89–2.61	1.49 (0.88–2.42)	1.62 (0.89–2.98)	1.02 (0.48–2.17)	1.55 (0.63–3.79)	0.94 (0.62–1.41)	0.84 (0.52–1.37)	1.19 (0.73–1.92)	1.27 (0.70–2.30)	1.05 (0.64–1.72)	1.02 (0.57–1.84)
≥2.62	1.44 (0.86–2.39)	1.66 (0.92–2.99)	1.50 (0.74–3.02)	**2.27 (1.01–5.11)***	0.99 (0.66–1.49)	0.95 (0.60–1.52)	0.87 (0.52–1.45)	0.92 (0.50–1.70)	0.77 (0.46–1.31)	0.76 (0.41–1.39)
D-limonene, μg/m³										
<12.19	1	1	1	1	1	1	1	1	1	1
12.19–30.49	0.98 (0.60–1.59)	1.04 (0.59–1.84)	1.04 (0.52–2.10)	1.04 (0.48–2.28)	1.23 (0.82–1.84)	1.31 (0.82–2.10)	**1.66 (1.02–2.70)***	**2.49 (1.35–4.59)***	1.42 (0.87–2.34)	**1.92 (1.06–3.47)***
≥30.50	1.05 (0.64–1.71)	1.01 (0.52–1.95)	0.98 (0.47–2.01)	0.98 (0.38–2.49)	0.93 (0.61–1.43)	1.03 (0.59–1.81)	0.93 (0.54–1.61)	1.62 (0.76–3.44)	0.89 (0.52–1.55)	1.10 (0.53–2.28)
α-pinene, μg/m³										
<1.00	1	1	1	1	1	1	1	1	1	1
≥1.00	1.55 (0.84–2.84)	1.82 (0.92–3.57)	1.79 (0.70–4.58)	2.63 (0.91–7.58)	0.87 (0.56–1.35)	1.00 (0.61–1.62)	0.98 (0.57–1.68)	0.92 (0.51–1.67)	1.14 (0.64–2.03)	1.17 (0.62–2.21)
TVOC, μg/m³										
<101.87	1	1	1	1	1	1	1	1	1	1
101.87–189.93	1.58 (0.96–2.61)	1.67 (0.93–3.00)	1.95 (0.89–4.26)	**2.56 (1.05–6.25)***	**1.50 (1.00–2.25)***	**1.65 (1.05–2.62)***	0.84 (0.51–1.38)	0.73 (0.40–1.32)	0.75 (0.47–1.27)	0.71 (0.39–1.29)
≥189.94	1.42 (0.86–2.32)	1.64 (0.94–2.85)	**2.22 (1.05–4.67)***	**2.67 (1.15–6.18)***	0.97 (0.64–1.48)	1.06 (0.67–1.68)	0.80 (0.49–1.29)	0.88 (0.52–1.49)	0.81 (0.50–1.32)	0.85 (0.50–1.45)
Formaldehyde, μg/m³										
<14.92	1	1	1	1	1	1	1	1	1	1
14.92–20.13	**1.65 (1.02–2.67)***	**2.11 (1.20–3.68)***	1.51 (0.79–2.91)	2.02 (0.95–4.29)	1.02 (0.68–1.54)	1.08 (0.68–1.74)	0.95 (0.58–1.56)	0.95 (0.54–1.70)	0.92 (0.56–1.52)	0.90 (0.51–1.61)
≥20.14	0.98 (0.58–1.68)	1.12 (0.56–2.24)	0.58 (0.25–1.34)	0.83 (0.30–2.31)	0.97 (0.64–1.48)	0.98 (0.57–1.68)	1.00 (0.61–1.64)	0.95 (0.48–1.86)	0.89 (0.53–1.49)	0.66 (0.33–1.32)
Acetaldehyde, μg/m³										
<5.80	1	1	1	1	1	1	1	1	1	1
5.80–9.82	1.34 (0.80–2.23)	1.59 (0.90–2.81)	1.59 (0.76–3.31)	1.60 (0.73–3.52)	0.88 (0.59–1.32)	1.07 (0.68–1.68)	0.92 (0.56–1.52)	0.89 (0.51–1.56)	0.83 (0.50–1.40)	0.80 (0.45–1.42)
≥9.83	1.60 (0.97–2.66)	**1.83 (1.01–3.33)***	1.46 (0.69–3.12)	1.92 (0.82–4.48)	0.89 (0.59–1.35)	0.98 (0.61–1.59)	1.02 (0.62–1.67)	1.14 (0.62–2.07)	1.09 (0.66–1.80)	1.12 (0.62–2.02)

OR: crude odds ratio; aOR: odds ratio adjusted for age, sex, mother's education, body mass index, relative humidity and temperature; CI: confidence interval; $^{*}p < 0.05$ (considered statistically significant) for the item in bold.

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 (Mendell, 2007; Gilbert et al., 2008). Taking into consideration that each classroom was equipped with standard plywood school furniture, and that currently no special care is taken regarding the household products used in the classrooms, a particular attention should be made regarding the selection of new furniture, cleaning, consumer and didactic products.

The indoor median concentration of $PM_{2.5}$ and PM_{10} in all of the classrooms exceeded the 25 μg/m³ and 50 μg/m³ guideline value suggested by WHO air quality guidelines for a sampling period of 24 h. The particulate matter concentrations measured in this study, in particular $PM_{2.5}$ levels, were higher than in previous studies performed in Europe, e.g. (Stranger et al., 2007; Almeida et al., 2011; Canha et al., 2015). According to Canha et al. (2014), $PM_{2.5}$ was studied in a Portuguese classrooms and the values were high

(100 ± 71 μg/m³) than those obtained in the current study. The authors pointed out to a high contribution of a specific indoor source that was a mixture of wood burning (for classroom heating), soil re-suspension and chalk. This could be the reason why PM_{10} is usually higher indoors than outdoors, while $PM_{2.5}$ is usually more related with outdoor infiltration in indoors (Almeida et al., 2011). 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). Fromme et al. (2007) reported that high PM_{10} levels in schools were correlated with less frequent cleaning and inefficient removal of deposited particles that consequently became re-suspended. This is

Table 3
Associations between particulate matter, bacteria, fungi and carbon dioxide and wheeze, nasal allergy, cough episodes and phlegm episodes (n = 978).

	Wheeze (<12 months)		Wheeze (<30 days)		Ever nasal allergy		Cough episodes		Phlegm episodes	
	OR (95% CI)	aOR (95% CI)	OR (95% CI)	aOR (95% CI)	OR (95% CI)	aOR (95% CI)	OR (95% CI)	aOR (95% CI)	OR (95% CI)	aOR (95% CI)
PM$_{2.5}$, µg/m^3										
<72.53	1	1	1	1	1	1	1	1	1	1
72.53—97.92	1.35 (0.81 −2.26)	1.72 (0.96 −3.08)	1.44 (0.64 −3.22)	1.44 (0.62 −3.39)	1.15 (0.77 −1.71)	1.25 (0.81 −1.92)	0.94 (0.56 −1.56)	1.18 (0.66 −2.09)	0.97 (0.58 −1.62)	1.11 (0.63 −1.96)
≥97.93	1.48 (0.88 −2.48)	**1.90 (1.04 −3.45)**[*]	**2.20 (1.02 −4.74)**[*]	**2.28 (1.00 −5.18)**[*]	0.79 (0.51 −1.22)	0.73 (0.45 −1.19)	1.26 (0.77 −2.07)	1.44 (0.81 −2.56)	1.08 (0.64 −1.81)	1.25 (0.70 −2.23)
PM$_{10}$, µg/m^3										
<116.77	1	1	1	1	1	1	1	1	1	1
116.77 −137.88	1.07 (0.64 −1.80)	1.23 (0.66 −2.27)	1.85 (0.78 −4.39)	1.58 (0.61 −4.09)	1.09 (0.73 −1.63)	1.24 (0.79 −1.95)	1.18 (0.70 −1.96)	1.19 (0.65 −2.18)	0.90 (0.54 −1.50)	0.89 (0.49 −1.61)
≥137.89	1.48 (0.90 −2.45)	**1.93 (1.07 −3.49)**[*]	**2.96 (1.30 −6.73)**[*]	**3.00 (1.23 −7.33)**[*]	0.79 (0.51 −1.21)	0.73 (0.44 −1.19)	1.36 (0.82 −2.27)	1.36 (0.75 −2.47)	1.05 (0.63 −1.74)	1.11 (0.62 −1.98)
Bacteria, CFU/m^3										
<2058	1	1	1	1	1	1	1	1	1	1
2058—3856	0.76 (0.48 −1.22)	0.83 (0.48 −1.41)	0.94 (0.49 −1.81)	0.88 (0.43 −1.83)	1.08 (0.72 −1.62)	1.29 (0.82 −2.04)	1.05 (0.61 −1.79)	1.33 (0.72 −2.46)	1.28 (0.74 −2.23)	1.44 (0.78 −2.64)
≥3857	0.61 (0.37 −1.01)	**0.51 (0.28 −0.92)**[*]	0.54 (0.25 −1.15)	0.52 (0.22 −1.19)	0.73 (0.47 −1.12)	0.72 (0.44 −1.19)	1.59 (0.96 −2.62)	**1.88 (1.03 −3.43)**[*]	1.73 (1.02 −2.94)	1.65 (0.90 −3.02)
Fungi, CFU/m^3										
<199.67	1	1	1	1	1	1	1	1	1	1
199.67—320	0.99 (0.62 −1.60)	0.87 (0.50 −1.51)	0.79 (0.41 −1.53)	0.89 (0.42 −1.89)	1.39 (0.92 −2.18)	1.54 (0.96 −2.47)	1.32 (0.80 −2.17)	1.39 (0.78 −2.47)	1.34 (0.81 −2.24)	1.20 (0.68 −2.14)
≥321	0.63 (0.37 −1.06)	0.57 (0.32 −1.01)	**0.36 (0.16 −0.83)**[*]	**0.38 (0.16 −0.91)**[*]	1.14 (0.74 −1.75)	1.20 (0.75 −1.93)	0.99 (0.58 −1.66)	0.87 (0.48 −1.58)	0.95 (0.55 −1.64)	0.78 (0.42 −1.41)
CO$_2$, ppm										
<1299.87	1	1	1	1	1	1	1	1	1	1
1299.87 −1913.07	0.62 (0.37 −1.06)	0.68 (0.36 −1.25)	1.15 (0.54 −2.47)	1.49 (0.64 −3.46)	0.97 (0.64 −1.48)	1.25 (0.76 −2.06)	**0.53 (0.31 −0.88)**[*]	**0.51 (0.28 −0.95)**[*]	**0.56 (0.33 −0.96)**[*]	**0.50 (0.27 −0.94)**[*]
≥1913.08	1.08 (0.67 −1.72)	1.19 (0.63 −2.26)	1.47 (0.71 −3.03)	2.07 (0.81 −5.27)	0.98 (0.65 −1.50)	1.30 (0.75 −2.26)	0.66 (0.41 −1.08)	0.85 (0.43 −1.68)	0.74 (0.45 −1.22)	0.70 (0.36 −1.37)

OR: crude odds ratio; aOR: odds ratio adjusted for age, sex, mother's education, body mass index, relative humidity and temperature; CI: confidence interval; [*]$p < 0.05$ (considered statistically significant) for the item in bold.

Table 4
Spirometry parameters values according to indoor air volatile organic compounds.

	FVC (% of the predicted value) (n = 761)			FEV$_1$ (% of the predicted value) (n = 761)			FEV$_1$/FVC (n = 761)			FEF$_{25-75}$ (% of the predicted value) (n = 749)		
	n	Mean (SD)	p-value	n	Mean (SD)	p-value	n	Mean (SD)	p-value	n	Mean (SD)	p-value
Toluene, µg/m^3			0.689			0.377			0.293			0.103
<4.64	257	103.9 (12.0)		257	107.2 (11.1)		257	103.5 (5.15)		254	108.9 (18.2)	
4.64—8.06	253	103.8 (11.6)		253	108.5 (11.9)		253	104.7 (5.35)		250	114.9 (22.6)	
≥8.07	243	102.4 (12.4)		243	106.0 (13.2)		243	103.6 (6.61)		237	109.1 (22.6)	
m/p-xylene, µg/m^3			0.075			0.205			0.414			0.939
<4.02	249	104.4 (11.6)		249	107.8 (10.7)		249	103.5 (5.46)		247	110.4 (19.2)	
4.02—5.89	261	104.6 (11.7)		261	108.4 (12.3)		261	103.8 (5.77)		257	111.0 (22.4)	
≥5.90	243	100.9 (14.4)		243	105.3 (12.8)		243	104.6 (5.82)		237	111.6 (21.8)	
o-xylene, µg/m3			0.728			0.808			0.125			0.221
<1.89	250	104.2 (11.8)		250	107.6 (10.8)		250	103.5 (5.51)		249	109.4 (18.4)	
1.89—2.61	247	102.8 (12.2)		247	107.7 (12.6)		247	104.9 (5.17)		245	114.2 (22.4)	
≥2.62	256	103.3 (12.0)		256	106.6 (12.6)		256	103.4 (6.27)		247	109.3 (22.3)	
ᴅ-limonene, µg/m^3			0.268			0.426			0.753			0.886
<12.19	254	104.8 (12.8)		254	108.3 (12.2)		254	103.6 (5.52)		250	111.9 (21.3)	
12.19—30.49	257	103.3 (11.7)		257	107.4 (12.0)		257	104.1 (4.89)		253	110.5 (19.4)	
≥30.50	242	107.8 (11.0)		242	105.9 (11.7)		242	104.2 (6.78)		238	110.6 (23.4)	
α-pinene, µg/m^3			0.459			0.601			0.640			0.903
<1.00	129	104.7 (10.0)		129	108.2 (9.80)		129	103.6 (5.73)		128	111.4 (19.5)	
≥1.00	624	103.2 (12.3)		624	107.1 (12.4)		624	104.0 (5.70)		613	111.0 (21.6)	
TVOC, µg/m^3			0.060			**0.013**[*]			0.534			0.158
<101.87	286	103.9 (12.9)		286	108.1 (12.3)		286	104.3 (5.49)		283	112.8 (20.7)	
101.87—189.93	214	105.1 (11.2)		214	109.2 (11.7)		214	104.0 (4.94)		210	112.4 (22.2)	
≥189.94	253	100.8 (11.1)		253	103.9 (11.5)		253	103.3 (6.73)		248	107.0 (20.6)	
Formaldehyde, µg/m^3			0.855			0.370			0.213			0.281
<14.92	267	102.9 (18.9)		267	106.2 (10.8)		267	103.4 (5.08)		266	108.6 (20.7)	
14.92—20.13	256	103.6 (11.9)		256	107.1 (11.7)		256	103.7 (6.75)		253	111.4 (21.0)	
≥20.14	238	103.9 (13.2)		238	108.7 (13.5)		238	104.8 (5.11)		230	113.7 (21.8)	
Acetaldehyde, µg/m^3			0.061			**0.029**[*]			0.471			0.432
<5.80	253	103.9 (12.1)		253	107.1 (11.3)		253	103.3 (5.41)		251	109.3 (19.4)	
5.80—9.82	279	101.4 (11.3)		279	105.3 (11.9)		279	104.0 (6.39)		273	110.7 (22.3)	
≥9.83	229	105.5 (12.2)		229	110.0 (12.4)		229	104.4 (4.99)		225	113.6 (21.5)	

FVC: Forced vital capacity; FEV$_1$: Forced expiratory volume in 1 s; FEF$_{25-75\%}$: Forced expiratory flow 25—75%; SD: Standard deviation; [*]$p < 0.05$ (considered statistically significant) for the item in bold.

Table 5
Spirometry parameters values according to indoor particulate matter, bacteria, fungi and carbon dioxide.

	FVC (% of the predicted value) (n = 761)			FEV$_1$ (% of the predicted value) (n = 761)			FEV$_1$/FVC (n = 761)			FEF$_{25-75}$ (% of the predicted value) (n = 749)		
	n	Mean (SD)	p-value	n	Mean (SD)	p-value	n	Mean (SD)	p-value	n	Mean (SD)	p-value
PM$_{2.5}$, µg/m^3			0.451			0.603			0.644			0.443
<72.53	224	102.2 (13.5)		224	106.5 (13.6)		224	104.4 (5.31)		217	111.0 (20.7)	
72.53–97.92	269	104.5 (11.2)		269	108.3 (10.9)		269	103.9 (5.80)		266	113.3 (22.5)	
≥97.93	262	103.5 (11.4)		262	107.1 (11.7)		262	103.6 (5.91)		260	109.3 (20.4)	
PM$_{10}$, µg/m^3			0.733			0.974			0.264			0.588
<116.77	230	102.5 (13.9)		230	107.2 (13.8)		230	104.8 (5.24)		222	112.0 (21.9)	
116.77–137.88	255	103.8 (11.0)		255	107.6 (10.7)		255	103.8 (5.64)		254	112.3 (21.1)	
≥137.89	270	103.8 (11.4)		270	107.2 (11.8)		270	103.4 (6.06)		267	109.4 (21.0)	
Bacteria, CFU/m^3			0.229			**0.026***			**0.032***			**0.002***
<2058.00	233	101.8 (10.4)		233	104.2 (10.4)		233	102.6 (5.36)		232	104.1 (18.0)	
2058.00–3856.00	260	104.9 (12.7)		260	109.1 (12.4)		260	104.3 (6.34)		252	114.8 (22.3)	
≥3857.00	252	103.0 (12.4)		252	107.8 (12.5)		252	104.8 (5.01)		249	113.4 (21.3)	
Fungi, CFU/m^3			0.200			0.268			0.762			0.747
<199.67	253	103.0 (12.3)		253	106.6 (12.5)		253	103.7 (5.36)		251	109.6 (22.2)	
199.67–320.00	226	101.8 (12.6)		226	106.0 (13.0)		226	104.3 (6.20)		224	111.6 (22.7)	
≥321.00	270	105.0 (11.0)		270	108.8 (10.5)		270	103.8 (5.61)		262	111.7 (18.8)	
CO$_2$, ppm			0.745			0.801			0.692			0.717
<1299.87	242	104.0 (10.4)		242	108.2 (9.79)		242	104.3 (4.69)		242	112.7 (17.5)	
1299.87–1913.07	243	102.0 (14.0)		243	106.2 (14.3)		243	104.3 (6.08)		236	111.3 (24.8)	
≥1913.08	249	103.9 (11.0)		249	107.4 (11.6)		249	103.5 (6.16)		245	109.6 (20.8)	

FVC: Forced vital capacity; FEV1: Forced expiratory volume in 1 s; FEF25-75%: Forced expiratory flow 25–75%; SD: Standard deviation; *$p < 0.05$ (considered statistically significant) for the item in bold.

consistent with the observations from other studies (Guo et al., 2010; Almeida et al., 2011; Oeder et al., 2012). Moreover, in Portugal, some studies already showed that there is a high contribution from the chalk used in the blackboards to the overall indoor PM$_{10}$ and total suspended matter (Almeida et al., 2011; Canha et al., 2014). 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. This illustrates the potential impact of the location of schools, and it could therefore be suggested that when new schools are built, outdoor risks factors should be taken into account.

Mean indoor levels of bacteria higher than 500 CFU/m^3 were observed in 63 (86%) classrooms; which is 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 3 schools in Lisboa also in winter time (Pegas et al., 2010). Indoor bacteria concentrations are higher when compared with outdoor levels, indicating significant indoor sources and insufficient ventilation. One possible cause for this observation may be the higher occupancy and active behavioural pattern/activity level of children in relatively small spaces. This assertion is also supported by a number of previous studies in which human occupancy was found to affect indoor microbial concentrations, as settled spores were re-suspended by human activities (e.g. walking and running in indoor sites) (Kim et al., 2007; Mentese et al., 2009). In addition these findings might also be due to poor ventilation taking into account that all schools are naturally ventilated as reported previously by Canha et al. (2013). Another possible explanation is the environmental conditions surrounding the buildings, as plants and soil outdoors; as well as the influence of season (winter time) and climate.

For both the indoor and outdoor air samples, the concentrations of fungi are lower than the concentrations of the bacteria, which are consistent with other studies (Godwin and Batterman, 2007). As the measurements were taken between November 2011 and March 2012, and between November and December 2012, the weather conditions included low outdoor temperatures (14.6 ± 3.3 °C) and

moderate precipitation levels, which may explain the lower outdoor concentrations. However, during the heating season, occupants generally spend more time in indoor environments, windows are more often closed and ventilation may be insufficient; thus, indoor temperature and relative humidity become suitable for fungal growth indoors. Indoor fungal growth is mainly affected by their outdoor concentration and indoor factors such, as temperature, humidity and building/furnishing materials (Meng et al., 2012). Indoor bioaerosol concentrations can be highly variable and influenced by many factors, e.g. the life cycle of the organism, season, humidity and window opening. Thus, the short-term bioaerosol samples reflect concentrations only for the day the samples were taken and may not be representative of long-term exposures.

In the present study, TVOC exposure levels were significantly associated with wheeze in the preceding month and were also related to increments in reported ever nasal allergy. Therefore, it can be supposed that TVOC may be associated with the presence of asthma or allergic rhinitis, although for the latter no nose symptoms were reported and the 3rd tertile of TVOC concentrations may reject the hypothesis of allergic rhinitis. Total VOC concentration was related to chronic airway and chronic eye symptoms by Hulin et al. (2010). Concerning spirometry, all parameters in the volume of exhaled air were lower where TVOC had higher concentrations (highest tertile), being statistically significant for FEV$_1$. The eNO results support the potential existence of chronic airway inflammation in children under medium TVOC exposure. In addition, eNO concentrations are significantly higher in children with symptoms of respiratory and allergic diseases when exposed to same TVOC concentrations as occurred with the remaining indoor air parameters. The potential mechanism of allergic sensitization could be the irritation properties of VOC. Volatile organic compounds could facilitate the penetration of allergens in the target organs by irritation of the respiratory mucosa and impaired muco-ciliary clearance (D' Amato et al., 2005). One of the molecular mechanisms that may explain the effects of indoor pollutants on inflammation is oxidative stress in which VOC may play a key role (Baeza and Marano, 2007; Bonay and Aubier, 2007). Another potential mechanism is a synergistic effect of sensitization and exposure to such pollutants on airway reactivity, lowering the dose of antigen

Table 6
Exhaled nitric oxide values according to indoor air parameters.

	eNO, ppb					
	Children without symptoms or disease[a] (n = 159)			Children with symptoms or disease[a] (n = 156)		
	n	Mean (SD)	p-value	n	Mean (SD)	p-value
Toluene, $\mu g/m^3$			0.626			0.474
<4.64	53	15.7 (19.8)		56	19.5 (18.4)	
4.64–8.06	60	13.4 (5.93)		50	23.4 (20.5)	
≥8.07	43	14.6 (7.69)		48	23.8 (21.7)	
m/p-xylene, $\mu g/m^3$			0.144			0.202
<4.02	53	12.9 (5.97)		48	21.1 (18.9)	
4.02–5.89	59	17.1 (19.2)		59	25.6 (22.8)	
≥5.90	44	12.9 (5.49)		47	18.7 (20.1)	
o-xylene, $\mu g/m^3$			0.507			0.725
<1.89	56	14.1 (6.39)		48	23.7 (21.3)	
1.89–2.61	56	13.4 (6.78)		50	22.3 (21.5)	
≥2.62	44	16.3 (21.6)		56	20.5 (17.9)	
D-limonene, $\mu g/m^3$			0.926			0.841
<12.19	63	14.7 (18.6)		49	21.6 (18.4)	
12.19–30.49	51	14.8 (6.03)		57	23.3 (21.5)	
≥30.50	42	13.8 (6.90)		48	21.1 (20.4)	
α-pinene, $\mu g/m^3$			0.731			0.622
<1.00	24	13.7 (5.61)		26	20.3 (16.8)	
≥1.00	132	14.6 (13.6)		128	22.4 (20.8)	
TVOC, $\mu g/m^3$			0.158			**0.012**[*]
<101.87	64	13.5 (6.82)		56	18.8 (16.7)	
101.87–189.93	45	17.6 (21.5)		52	28.8 (24.8)	
≥189.94	47	13.0 (5.21)		46	18.6 (16.3)	
Formaldehyde, $\mu g/m^3$			0.193			0.061
<14.92	58	13.4 (4.99)		56	24.8 (22.7)	
14.92–20.13	48	17.4 (20.8)		55	24.4 (20.2)	
≥20.14	53	13.4 (7.56)		45	16.2 (15.0)	
Acetaldehyde, $\mu g/m^3$			0.354			0.358
<5.80	53	12.9 (4.73)		52	25.4 (22.4)	
5.80–9.82	55	16.40 (19.4)		57	20.3 (17.9)	
≥9.83	51	14.4 (8.32)		47	20.8 (19.8)	
$PM_{2.5}$, $\mu g/m^3$			0.212			0.210
<72.53	51	17.0 (20.6)		46	19.6 (16.9)	
72.53–97.92	53	12.7 (4.74)		58	20.8 (19.3)	
≥97.93	53	14.1 (6.78)		50	26.4 (23.5)	
PM_{10}, $\mu g/m^3$			0.430			0.165
<116.77	51	16.5 (20.7)		48	18.9 (18.3)	
116.77–137.88	53	13.4 (4.72)		55	21.4 (18.4)	
≥137.89	53	14.0 (6.92)		51	26.4 (23.2)	
Bacteria, CFU/m^3			0.432			0.060
<2058.00	49	12.8 (5.49)		49	27.6 (23.0)	
2058.00–3856.00	54	16.1 (19.6)		55	21.1 (19.0)	
≥3857.00	54	14.9 (7.81)		49	18.1 (17.7)	
Fungi, CFU/m^3			0.881			0.645
<199.67	51	14.2 (5.58)		52	24.3 (22.1)	
199.67–320.00	49	14.2 (7.36)		51	22.1 (21.0)	
≥321.00	58	15.2 (19.2)		50	20.6 (17.5)	
CO_2, ppm			0.466			0.369
<1299.87	55	13.4 (5.34)		51	25.1 (20.1)	
1299.87–1913.07	55	16.2 (19.5)		53	19.5 (18.6)	
≥1913.08	43	14.0 (7.40)		50	21.9 (21.7)	

SD: Standard deviation; [a] Disease diagnosed by a doctor; [*]$p < 0.05$ (considered statistically significant) for the item in bold. Note: Kruskal–Wallis test was used to compare eNO values and indoor air parameters.

exposition needed to provoke bronchial or nasal constriction (Roux et al., 1999; Leikauf, 2002). Given the potential impact of exposure to VOC on children's health, it is important to increase the understanding of the factors that affect their indoor concentration. According to Mendell (2007) and Zhang et al. (2006), indoor TVOC levels might be due to the furnishing, floor covering, insulating materials, adhesives, paints and glues as well as other solvents and cleaning products. In addition to these indoor sources, the insufficient ventilation is likely to favour the increase of TVOC levels (Zhang et al., 2006) underlining the importance of occupant behaviours in the control and guarantee of healthy indoor air.

Formaldehyde is one of the most studied pollutants when focussing on the effect on indoor air pollution on respiratory health. According to Mendell (2007), recent epidemiological studies confirm the allergic potential of formaldehyde in the development of asthma and other allergic symptoms. A recent meta-analysis focussing on formaldehyde exposure and asthma in children calculated a pooled OR of 1.03 (95% CI: 1.02–1.04) for an increase of 10 $\mu g/m^3$ (McGwin et al., 2010). Two studies focused on respiratory effects of indoor formaldehyde exposure revealed an increased risk of asthma associated with elevated indoor concentrations (Smedje and Norbäck, 2001; Rumchev et al., 2002). In the present study, it was found that indoor exposure to formaldehyde was related to asthma-like symptoms, namely, wheeze in the past year and irritating cough reported during the previous 3 months. However, Ezratty et al. (2007) and Billionnet et al. (2011) questioned the effect of formaldehyde on asthma. As suggested by Wolkoff and Nielsen (2010), complex co-exposures with other compounds, exposure levels and socio-economic factors could encumber the interpretation of the association with formaldehyde, explaining different findings among studies. Acetaldehyde was also found to be associated with asthma-like symptoms in our population. In agreement with our findings, exposure to acetaldehyde at home, in a subsample of the 6 Cities study, was associated with a higher risk of asthma (Hulin et al., 2010). So far, few studies have assessed the effects of school indoor air parameters on children health using clinical objective measurements (Kim et al., 2007; Zhao et al., 2008; Le Cann et al., 2011; Martins et al., 2012). In the specific case of the present study, no associations were obtained between specific aldehydes and the inflammatory indicator eNO. Therefore, in light of the evidence that the variations in eNO measurements show some inconsistencies in children exposed to environmental pollutants, further research appears to be needed.

An increasing number of data has shown that increased levels of $PM_{2.5}$ and PM_{10} should result in the increased prevalence of acute and chronic health effects, including asthma, among children (Daisey et al., 2003; Mendell and Heath, 2005). Links between health and $PM_{2.5}$ concentrations were published for schoolyards and for classrooms (Annesi-Maesano et al., 2012). The present study showed that higher levels of $PM_{2.5}$ and PM_{10} increase the odds of asthma-like symptoms (wheeze and irritating cough) among children; nevertheless the association was stronger for PM_{10}. However, for the irritating cough this relationship disappears when the children with "feeling like getting a cold" are excluded. Annesi-Maesano et al. (2012) reported that high prevalence of asthma in the past year was found in children attending classrooms with $PM_{2.5}$ levels higher than 17.5 $\mu g/m^3$. It was also reported that increased PM_{10} concentrations had significant pertinence on lung disorders (such as wheeze and shortness of breath) and on reduction of lung functions; while $PM_{2.5}$ were strongly associated with cardiopulmonary diseases and lung cancer (Gemenetzis et al., 2006). In addition, spirometry did not reveal an obstructive pattern. Nevertheless, given the association between wheeze symptoms, $PM_{2.5}$ and PM_{10} levels, as well as the eNO results that, although not significant, were higher in the 3rd tertile among the children with symptoms or disease diagnosed by a doctor, it is possible to point out that particulate matter is associated with an increase in airways inflammation. Most studies of particulate matter have been focused on ambient (outdoor) exposures and their relationship to hospital admissions and mortality (Jaakkola et al., 2000). Although the mechanisms are not well understood, it has been shown that particulate matter may enhance airway inflammatory reactions and sensitization (Jaakkola et al., 2000). It has been suggested that this could be due to the intervention of the polycyclic aromatic hydrocarbon contained in particulate matter or strongly related to it. However, it cannot be excluded that

particulate matter might also influence non-immunological properties of the allergens, such as their enzymatic activity, thus contributing to their increased penetration in the target organs (Steerenberg et al., 2003). Since the classrooms do not contain any specific $PM_{2.5}$ source (such as smoking, cooking), the indoor $PM_{2.5}$ concentrations were more likely to be due to outdoor penetration rather than indoor sources related to the presence of children and the intensity of their indoor activities as reported by Polidori et al. (2007); as well as resulted from reactions between ozone and terpenes (Sarwar et al., 2003; Weschler and Shields, 2003).

Regarding the exposure to indoor biological agents and after adjustment, lower odds of wheeze in the previous year were found among those in schools with higher levels of bacteria and of wheeze in the previous month among those in schools with higher levels of fungi. By contrast, higher levels of bacteria were significantly associated with higher odds of cough episodes. This is in contrast with a previous school study reporting positive associations between indoor viable bacteria measured in spring and current asthma (wheeze in the past 12 months) (Smedje et al., 1997) as well as with exacerbation of asthma (Douwes et al., 2003). Similarly, the findings of most epidemiological studies on bacterial components (such as endotoxin) have been contradictory (Radon, 2006). Some demonstrated protective effects, while others showed associations with increased asthma symptoms in children (Michel et al., 1996; Michel, 2000). In addition, despite the fact that spirometry parameters in general showed little variation, it was still possible to verify a significant difference in $FEF_{25-75\%}$ which was lower with higher levels of bacteria. The association between the concentrations of bacteria and the eNO results was more pronounced in children with symptoms of respiratory and allergic disease than in asymptomatic children, although the difference was not significant. According to Mendell et al. (2011), quantitatively determined concentrations of microbiological agents do not show a consistent association with respiratory health outcomes; in some cases, exposure to microbial factors is protective against asthma-related symptoms and wheezing, particularly for those who are exposed very early in life (Mendell et al., 2011). The inconsistent association between exposure to bacteria/fungi and health outcomes in different studies could in part be due to variations in study design, sampling and analysing method, season of the year for the measurements, region where the measurements have been made, climate and indoor activities, etc. (Ren et al., 2001; Chew et al., 2003). Additionally, the culture method has been criticised because of the short-term sampling of the measurement, which could not effectively represent the time-averaged conditions experienced by children (Pasanen, 2001). There are also limitations in culture-based sampling methods for characterizing health-related bioaerosol composition and concentration indoors (Pasanen, 2001). However, several fungi produce allergens known to be associated with allergies and asthma; many fungi also produce toxins and irritants with suspected effects on respiratory health (WHO, 2009). Stark et al. (2005) noted that total fungi concentrations would group diverse genera into a single exposure variable that may not accurately predict risk. In addition, Holme et al. (2010) found no significant association between fungi concentrations and health outcomes despite the significant associations with specific genera. Fungi species of *Penicillium*, *Aspergillus* and *Cladosporium* have been the most frequently associated with allergy and exist both in indoor and outdoor environments (Daisey et al., 2003; Jo and Seo, 2005). This could imply that measurements of specific genera predict health outcomes better than viable fungi concentrations. Several aspects of this study are noteworthy as strengths. This study provides an IAQ investigation in a larger number of classrooms of public primary schools located in Porto and collected detailed information

on health, based on questionnaire and clinical tests, of a susceptible population. In addition, measurements were performed for a broad spectrum of chemical, physical and biological agents in classrooms allowed a better appraisal of individual exposure compared to indirect methods such as the exclusive use of questionnaires or checklists (Viegi et al., 2004). Moreover, detailed health data on a large number of children (participation rate of about 70%) were collected in the questionnaire study. Additionally, the use of different objective clinical outcomes is also a point that should be emphasised.

Because of the cross-sectional design of the study, the findings do not allow causal relationships between indoor air parameters and health outcomes. Although indoor air measurements were avoided during vacations or weekends and the school staff and teachers were asked not to modify their activities and behaviour during the survey to reduce misclassification, the measurement conducted during a single week may be a poor surrogate for past months/year exposure. Furthermore, one problem is selection bias, when i.e. parents of allergic children might be more willing to respond to questionnaires focussing on asthma and allergy compared with parents of non-allergic children (Bornehag et al., 2012). Furthermore, parents of children with allergies may also answer systematically differently compared with parents of children without allergy i.e. reporting bias. Another limitation may be associated with a minor contribution from the outdoor and home air exposure to the development of asthma-related symptoms. Also, the statistical analysis was performed using a 2 level factorial designs (child←classroom) and not 3 levels (child←classroom←school). Minimizing all these potential biases and, as well, increasing the response rates are known ways to obtain good data quality in questionnaire studies (Morton et al., 2006). Although the participation rate of 69.2% was reasonably high, the possibility of selection bias should still be considered.

This study contributes with new information on school environments in Portugal and their relationship with children health. This knowledge will be useful to develop appropriate control strategies and, thus, to limit exposures that may cause or contribute to asthma, allergy and other respiratory symptoms in children.

5. Conclusions

This work conducted a comprehensive characterization of a vast array of indoor air pollutants in 73 classrooms and investigates its relationship with respiratory symptoms among children aged 8—10 years. The exposure levels for the most indoor air parameters in the schools in the current study are in accordance with IAQ guidelines/recommendations, except for particulate matter and bioaerosols; however exposure to indoor air pollutants, especially higher levels of TVOC, $PM_{2.5}$ and PM_{10}, could increase self-reported respiratory symptoms among children.

Children exposed to higher TVOC concentrations had a twofold increased risk of having asthma-related symptoms. These findings were supported by the results of spirometry and eNO tests which suggested the existence of chronic airway inflammation. Moreover, higher $PM_{2.5}$ and PM_{10} levels increase the odds of asthma-like symptoms, with stronger association for PM_{10}. The present study supports the pro-inflammatory role of $PM_{2.5}$ and PM_{10}, especially among more susceptible children.

Taking into account the unique characteristics of the public primary schools, such as large number of children who are more susceptible to air pollutants, time spend indoors, higher density of occupation — there is a need to develop appropriate control strategies to minimize the adverse health effects on children, teachers and school staff.

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Appendix A. Supplementary material

Supplementary material related to this article can be found at http://dx.doi.org/10.1016/j.atmosenv.2015.07.028.

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