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Exploring the possibility of using CO₂ as a proxy for exhaled particles to predict the risk of indoor exposure to pathogens

Dadi Zhang and **Philomena M Bluyssen**

Abstract

Airborne transmission has been confirmed as one of three principal ways of SARS-CoV-2 transmission. To reduce the transmission of SARS-CoV-2 indoors, understanding the distribution of respiratory droplets (or aerosols) present in human breath seems therefore important. To study whether the CO_2 concentration can be used as a proxy for the number of exhaled particles present in an occupied space, the distribution of particles with different diameters (0.3, 0.5, 1.0, 2.5, 5.0 and 10 μm) and CO_2 concentrations were monitored in a classroom setting with six healthy subjects. Additionally, numbers of particles with the same sizes were measured in the breath of the same six healthy subjects separately. Results showed that (1) on the contrary to CO_2, the main source of indoor particles came from outdoor air, and not from occupants; (2) the impacts of ventilation regimes on indoor particle numbers were different to the impacts on CO_2 concentrations; and (3) almost no significant relationship between the number of indoor particles and CO_2 concentration was observed. Based on these results, this study could therefore not conclude that the CO_2 concentration in a classroom can be used as a proxy for the number of exhaled particles by the occupants.

Introduction

Airborne transmission of SARS-CoV-2 is one of the three transmission routes, deemed to be responsible for the global pandemic of coronavirus disease 2019 (COVID-19).[1,2] A broad range of exhaled particles can be transmitted by exhaling, sneezing, coughing, talking and singing through (1) direct transmission of larger droplets that directly fall on the mouth, eyes or nose of other people; (2) fomites (or indirect) transmission, droplets that fall on surfaces which are touched by other people; and (3) airborne transmission of aerosols, at close-range or long-range which are inhaled by other people.[3–5] To cope with the direct and indirect transmission, keeping social distance, washing hands and wearing masks were implemented. To reduce airborne transmission, sufficient ventilation measures have been recommended by many researchers and international organizations.[4,6–8] However, since the behaviour and distribution of exhaled particles are difficult to measure and predict, it is still unknown how much should be ventilated in order to reduce the risk of airborne transmission.

Determining how much ventilation is required and how it is ventilated, is particularly important for school classrooms because of the high density of school children and consequently a higher risk of airborne transmission.[9]

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Since classrooms are usually crowded and poorly ventilated,[10] they were seen as high-risk environments for children during the COVID-19 pandemic. To ensure the children's health, most schools were closed during the lockdown.[11] Unfortunately, this measure caused severe health effects among school children, including both physical symptoms and mental disorders.[12] To avoid these effects, schools need to be reopened, and to keep a healthy environment, improving the ventilation was one of the major measures taken by school managers.11 However, the amount of ventilation is still difficult to determine because current guidelines are set up to prevent high CO_2 concentrations, not airborne transmission.[13]

The virus SARS-CoV-2 has a size of around 120 nm (0.12 μm) in diameter. During breathing, talking, sneezing, etc., the virus is aerosolized and encapsulated in water-based particles with a size ranging from <1 μm to >100 μm.[14–16] The behaviour and pathway of these pathogen-loaded particles depend on their size and weight. To reduce airborne transmission with ventilation, it is important to know how these particles with different sizes behave and distribute, and which sizes of particles are most important. Many studies have been conducted to establish relationships between the SARS-CoV-2 and particles of different sizes. Results of these studies varied a lot due to different sampling methods and instruments. Liu et al.[17] found that the SARS-CoV-2 virus was mainly associated with particles of a diameter between 0.25 and 1.0 μm. Nor et al.[18] observed a relationship between $PM_{2.5}$ (particles with a diameter less than 2.5 μm) generated by people and the presence of SARS-CoV-2, and thus suggested that $PM_{2.5}$ could be a transport agent of SARS-CoV-2. Chia et al.[19] detected the virus in particles with a diameter between 1 and 4 μm. It seems that all particles with a diameter less than 5.0 could be a potential carrier of SARS-CoV-2, similar as the conclusion drawn by Fennelly,[20] that although the size range of infectious particles produced by people is quite wide, the pathogens were mainly found in small particles (diameter <5 μm).

To better understand the behaviour of exhaled particles and to predict the infection risk of pathogens, CO_2 has been proposed as a surrogate of these particles, because both of them are ingredients of human breath and CO_2 is relatively easy to measure.[21] In several previous studies, CO_2 concentration has been used to predict the risk of airborne transmission.[21–23] Most of these studies were based on theoretical deductions. For example, Hartmann and Kriegel[21] derived an equation to establish a relationship between CO_2 and aerosol concentrations and thus calculated the aerosol concentration using the measured CO_2 concentration. However, this relationship was established under several assumptions, for example, CO_2 and aerosol concentrations were steady and well-mixed, which in practice is hardly achieved.[24] To avoid such assumptions, Rudnick and Milton[23] came up with a non-steady-state CO_2-based risk equation, which resulted in a relationship between the CO_2

concentration and the infection risk. They used the CO_2 concentration, as a marker of exhaled breath, to calculate the rebreathe rate (the percentage of inhaled air that was exhaled by other people previously in the same space) which could be applied to indicate the risk of infection. Besides the theoretical calculation, computational fluid dynamics (CFD) modelling is another commonly used method to study the distribution of particles.[25] He et al.[26] predicted the transmission behaviour of particles with diameters of 0.8 μm, 5 μm and 16 μm as well as a tracer gas (as a surrogate for fine particles with diameters less than 1 μm) under different ventilation regimes using CFD simulations. They found a similar concentration distribution for tracer gas and particles with diameters of 0.8 μm and 5 μm. However, according to Wang et al.,[27] uncertainties about the boundary conditions and transient models exist when applying CFD simulations and these uncertainties might cause errors. Thus, to get more precise results, a full-scale experiment is recommended. Bivolarova et al.[28] conducted a series of measurements in a test chamber with mixing ventilation to investigate the possibility of using tracer gas to simulate the behaviour of particles and concluded that behaviours of the tracer gas and fine particles were similar. However, in this study, only one unheated manikin was used instead of real people, as the source of particles and tracer gas. The complexity of the human body shape and the emission rate and concentration of the gas and particles generated by human breathing might lead to certain errors. Apart from that, most previous full-scale experiments were conducted under mechanical ventilation in the setting of a hospital room/office room/cabin,[28–30] while natural ventilation (which is commonly used in school classrooms[6]) in a classroom setting with real persons sitting inside has rarely been considered. As discussed in the recently issued *ASHRAE Position Document on Indoor Carbon Dioxide*,[31] using CO_2 as the indicator of airborne infection risk transmission relies on many assumptions and might not be highly accurate. Therefore, to verify whether CO_2 concentration can be used as a proxy for particle numbers (especially the smaller ones) in classrooms, a full-scale experiment under different ventilation regimes with real occupants is still required.

Considering all the above-mentioned research gaps, this study aimed to verify whether the CO_2 concentration can be used as a proxy for the number of exhaled aerosols in a classroom setting. To answer this question, in this study, (1) the numbers of particles with different sizes both in different people's breathing zone and in outdoor air were measured; (2) the distributions of particles with different sizes and CO_2 concentration in a classroom setting under different ventilation regimes, were determined; and (3) possible relationships between CO_2 concentration and the number of monitored particles with different sizes were tested.

Table 1. Study design.

Experiment	Time	Room*	Ventilation	Subject
Number of particles with different sizes and CO_2 concentrations in different people's breathing zones	9:50–10:00 10:00–10:09 10:11–10:19 10:21–10:29 10:31–10:39 10:41–10:49	Test chamber	Mixing ventilation (400 m³/h)	No (background) Subject 1 Subject 2 Subject 3 Subject 5 Subject 6
Distribution of particles and CO_2 concentration in a classroom setting	11:00–12:10	Experience room	Mixing ventilation (600 m³/h)	Subjects 1–6
	12:10–13:20	Experience room	Mixing ventilation (1200 m³/h)	Without subjects
	13:20–14:50	Experience room	Nature ventilation (450 m³/h)	Subjects 1–6
Number of particles with different sizes and CO_2 concentrations in different people's breathing zones	14:52–15:01 15:02–16:01 16:12–16:21 15:23–15:32 15:34–16:43 16:46–16:55 16:59–17:08 17:10–17:19	Test chamber	Mixing ventilation (400 m³/h)	No (background) Subject 1 Subject 2 Subject 3 Subject 4 No (background) Subject 6 No (background)

Note: * the temperature and relative humidity in the test chamber/Experience room were 23°C and 43%, respectively, during the experiment.

Methods

Study design

Two different experiments, including the measurement of (1) the number of particles with different sizes in different people's breathing zone and (2) the distribution of particles with different sizes and CO_2 concentrations in a classroom setting, were included in this study. Six healthy subjects, including one male and five females, participated in both experiments in the SenseLab.[32] All participants were in good health and were non-smokers. The average age of them was 35 (median 30) years old. There was no potential source of outdoor air pollutants nearby (within 100 metres) that might have influenced the IAQ in the SenseLab. According to the data reported by the local weather station, the outdoor conditions on the experimental day were 16°C, 63% and with 13 km/h Northern wind.[33] The time schedule is presented in Table 1.

Particles and CO_2 measured in different people's breathing zone

The first experiment was performed in one of the test chambers of the SenseLab (width 2.4 m, length 3.9 m, height 2.1 m). Six subjects participated in this experiment, one after another. All subjects were employees of the Delft

University of Technology and in good health. They were instructed to breathe normally towards a box (see Figure 1) where a particle counter (TROTEC PC220, proven to be a reliable instrument to investigate the concentration of aerosol particles[34]) was placed at 20 cm distance from the edge. This spot was selected to make sure the device was located directly in the exhaled air stream.

Only breathing was taken into consideration in this study because breathing occurs much more frequently than coughing or sneezing. The door was closed and only one subject was inside the chamber during the measurement. The measuring period was set at 10 minutes for each subject, and both devices measured and recorded the data once per minute. The particle counter has six size channels (10 to 5.0 μm, 5.0 to 2.5 μm, 2.5 to 1.0 μm, 1.0 to 0.5 μm, 0.5 to 0.3 μm and below 0.3 μm) with a counting efficiency of 50% for the 0.3 μm channel and 100% for particles in all the other channels. The measurement ranges for the six channels (from largest to the smallest sizes) are 0–340, 1–966, 0–2724, 0–41,600, 0–175,000 and 0–500,000, respectively. The working principle of the particle counter is light scattering which measures both liquid and solid particles.[35] This device was manually calibrated before the experiments.

This experiment was conducted twice, in the morning and in the afternoon, for each subject, with the exception of subject 4 who did not participate in the morning, and subject
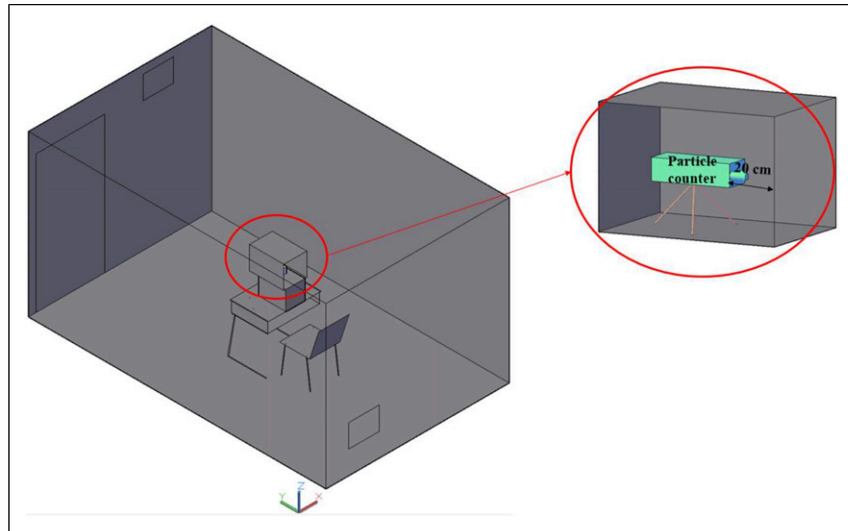
Figure 1. Experimental set-up in the test chamber of the SenseLab.

Notes: 1. 1-6 represent six measurement points; 2. During the experiment, subjects 1-6 were sitting on the six chairs next to the points 1-6, correspondingly.
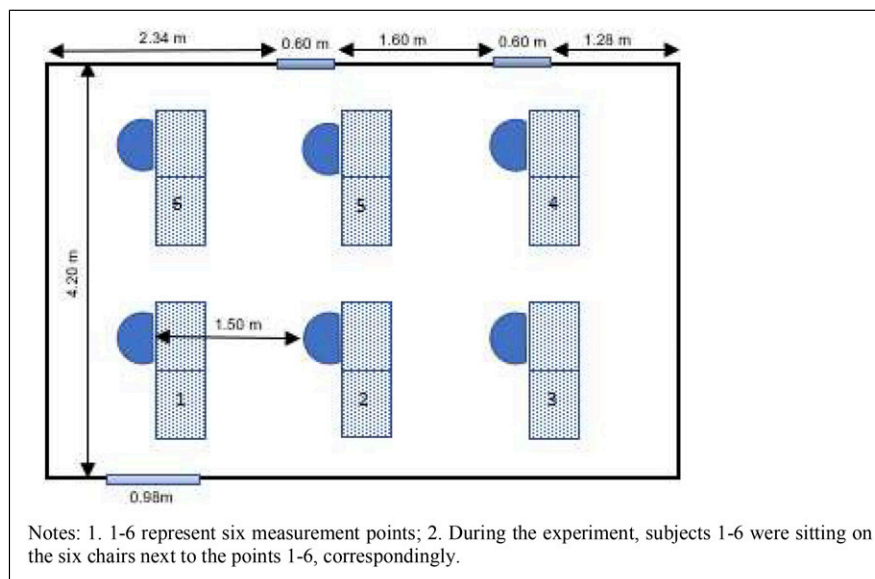
Figure 2. Experimental set-up in the Experience room of the SenseLab. Notes: 1.1–6 represent six measurement points; 2. During the experiment, subjects 1–6 were sitting on the six chairs next to the points 1–6, correspondingly.

5 who did not participate in the afternoon. Apart from the indoor measurements, the number of particles was measured outdoors (without subjects) with the same device and the same time interval and duration.

Particles and CO_2 distribution in a classroom setting

The second experiment was performed with six subjects sitting in the Experience room of the SenseLab (width 4.2 m, length 6.5 m, height 2.6 m) with 1.5 m between each

other, shown by the six chairs in Figure 2. The Experience room, located on the ground floor, has two openable windows (0.6 × 1.0 m; facing north) and one interior door (1.0 × 2.7 m; connected to the entrance hall of the SenseLab). The layout of it was similar to a classroom. Six measurement points were selected in the room (numbers '1'–'6' in Figure 2) with a distance of 20–30 cm from the mouth of each subject. The measurements were conducted at each point for 10 minutes, with a time interval of 1 minute. The particle counter used was the same as in the first experiment, next to which, a CO_2 logger (HOBO®

MX1102) was placed to monitor the CO_2 concentration, temperature (T) and relative humidity (RH). The HOBO had an accuracy of ±50 ppm in the range of 0–5000 ppm for CO_2 concentration, an accuracy of ±0.2°C in the range of 0–50°C for T, and an accuracy of ±2% in the range of 1–70% for RH. Because only one set of devices was available, the measurements at the different points were conducted after each other. Because natural ventilation and mixing ventilation are the most commonly used ventilation regimes in Dutch school classrooms,[36] to keep a healthy experimental environment during the COVID-19 pandemic, and to meet the ventilation rate required by ASHRAE,[37] '600 m^3/h mixing ventilation (with 100% outdoor air)' and 'natural ventilation with both windows and door open (with a ventilation rate of approximately 450 m^3/h)' were selected as the ventilation regimes when subjects were sitting inside the Experience room. Additionally, between these two tested conditions, to clean the Experience room from particles and CO_2 generated by the subjects, 1200 m^3/h mixing ventilation was applied when no subjects were inside the room (see Table 1).

Data analysis

All collected data were imported and analysed in four steps using SPSS version 26.0 (SPSS Inc. Chicago, IL, USA). For the data collected from people's breathing zones in the test chamber, a four-step statistical analysis was performed. First, the basic information (e.g. the mean and standard deviation of these parameters) was analysed with descriptive analysis, and normal distributions of all the parameters (particle numbers at different frictions) were checked using both Kolmogorov–Smirnov test and Shapiro–Wilk test.[38] Second, the difference in the number of particles measured in the breathing zone between two time periods was compared for each subject with paired samples t-tests.[39,40] Then, the differences between different subjects were compared with one-way ANOVA.[40] Finally, to compare the difference between the tests with and without subjects, all results measured in the subjects' breathing zones were compared with the results measured in the situation without subject, using paired samples t-tests.[40]

For the experiment conducted in the Experience room, similarly, at the beginning, the basic information (e.g. the mean and standard deviation of the measured number of particles and CO_2 concentration) was analysed, and normal distributions of all the parameters (particle numbers, CO_2 concentrations, T and RH) were checked using both Kolmogorov–Smirnov test and Shapiro–Wilk test under different experimental conditions.[38] Then, the differences in the number of particles between the six locations were compared using independent one-way ANOVA. After that, differences in the number of particles between every two of the three conditions were compared with paired samples

t-tests. Finally, the relationships between all measured parameters (number of particles, CO_2 concentration, T and RH) were tested using Pearson correlations.[39]

Ethical aspects

The subjects were asked to give informed consent to participate in the experiment. They were able to leave the test chamber/Experience room at any time in the case they were not feeling comfortable.

Results

Particles measured in different people's breathing zone

Figure 3 shows the average particle numbers measured in the subjects' breathing zones for 10 minutes. Results are shown in six fractions: 10 to 5 μm, 5 to 2.5 μm, 2.5 to 1.0 μm, 1.0 to 0.5 μm, 0.5 to 0.3 μm and below 0.3 μm. Similar size distributions of particles were found in all subjects' breathing zones. The majority was always the particles with diameter less than 0.3 μm, namely, $PM_{0.3}$, and the numbers of particles decreased with an increase in their sizes.

Generally speaking, the numbers of particles measured in the subjects' breathing zones were much higher than in the indoor background (see Figure 3(a) and (b)), and the average numbers of particles outdoors were much higher than indoors, with or without subjects (see Figure 3(c) and (d)).

Differences in particles in a person's breathing zone between morning and afternoon. To test whether the number of particles in the same subject's breathing zones differs during the day, a series of paired samples t-tests were applied to compare the number of particles in the breathing zones of four subjects (who participated in the tests twice) between in the morning and in the afternoon. Beforehand, all particle numbers in different fractions and under different settings, except for particles in the background setting and particles with diameters larger than 2.5 μm, were tested to be normally distributed. Since both paired t-test and one-way ANOVA were proved to be a robust test, modest departures from normality will not have much impact on the result, especially for the groups with equal sizes.[41,42] As shown in Table 2, no significant difference in the background number of particles between the two tests was found. However, there were significant differences of the number of particles generated by the subjects from breathing between the morning and the afternoon. Generally speaking, subjects produced more particles in the morning than in the afternoon, but the differences were found in different particle sizes for different subjects. For subjects 1 and 3, a
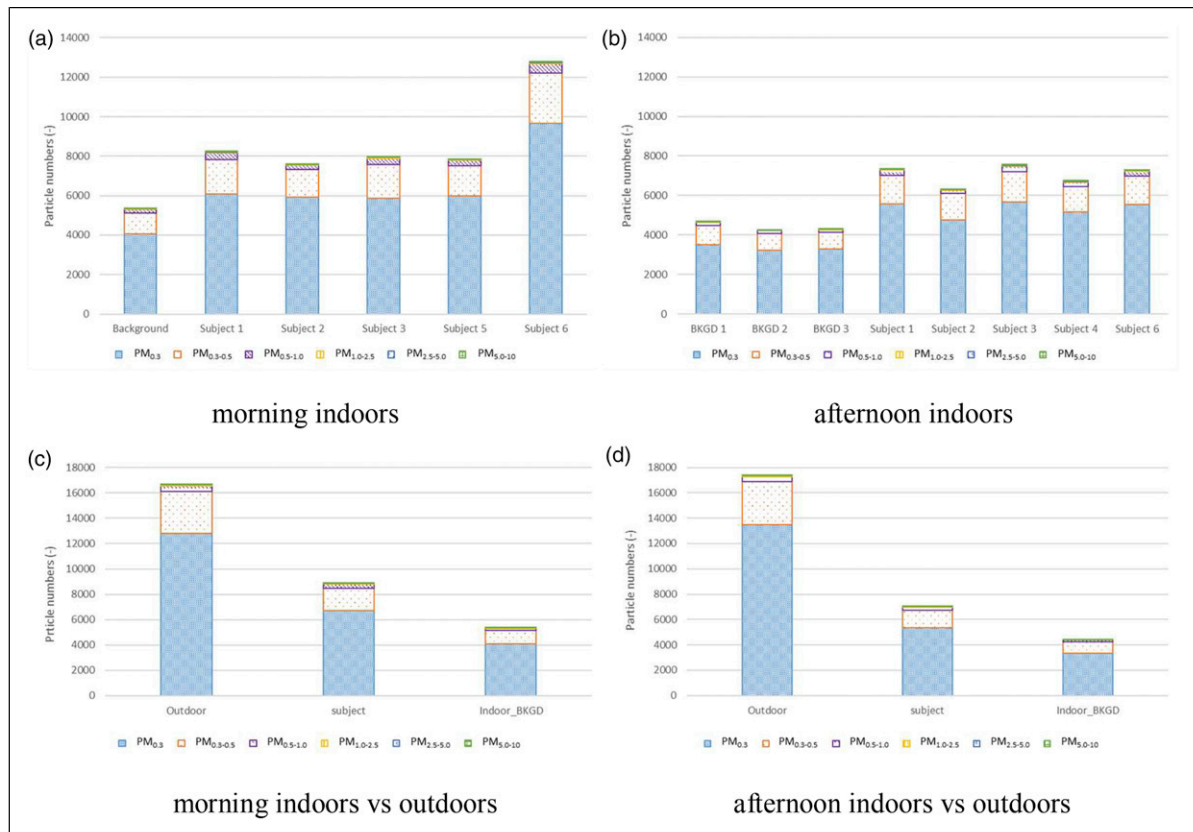
Figure 3. Average numbers of particles measured per fraction in different settings: (a) morning indoors; (b) afternoon indoors; (c) morning indoors versus outdoors; (d) afternoon indoors versus outdoors.

Table 2. Differences of the number of particles per subject and per fraction between morning and afternoon.

	$PM_{0.3}$	$PM_{0.3-0.5}$	$PM_{0.5-1.0}$	$PM_{1.0-2.5}$	$PM_{2.5-5.0}$	$PM_{5.0-10}$
Background	554.2 (0.172)	84.4 (0.366)	11.2 (0.665)	−0.4 (0.925)	−1.2 (0.350)	−1.7 (0.301)
Subject 1	508.2 (0.205)	**330.9 (0.045)**	32.3 (0.532)	3.0 (0.629)	3.8 (0.112)	−0.1 (0.946)
Subject 2	**1145.9 (0.046)**	76.4 (0.768)	**76.7 (0.014)**	5.4 (0.260)	1.6 (0.238)	−0.3 (0.708)
Subject 3	188.4 (0.526)	**187.4 (0.032)**	27.1 (0.579)	4.2 (0.683)	−0.6 (0.865)	0.2 (0.879)
Subject 5	**4119.1 (0.000)**	**1114.5 (0.000)**	**214.6 (0.001)**	**43.0 (0.001)**	**7.0 (0.004)**	1.9 (0.097)

Notes: 1. The results were obtained from paired samples t-tests; 2. The numbers shown in the table are the differences in the number of particles measured in the two tests; 3. The numbers in the parentheses are the *p*-values, and *p*-values less than 0.05 are marked in bold.

significant difference was found in $PM_{0.3-0.5}$; for subject 2, it was found in $PM_{0.3}$ and $PM_{0.5-1.0}$; while for subject 5, it was found in almost all the particle sizes, except for $PM_{5.0-10}$.

Differences in the number of particles between different subject. Table 3 lists the average numbers of particles measured in subjects' breathing zone and the results of one-way ANOVA, indicating the differences in the number of particles between different subjects. As it is shown, the differences were more obvious in the morning

than in the afternoon. According to a Bonferroni test, in the morning test, the differences between the subjects were all caused by the significantly high number of particles generated by subject 5. No significant differences were found between the other four subjects. For the afternoon test, almost no significant differences were found between the number of particles generated by the subjects, except for $PM_{0.5-1.0}$. Based on a Bonferroni test, the differences mainly exist between subjects 1 and 2, and between subjects 2 and 3.

Table 3. Average number of particles measured in subjects' breathing zone in the morning and in the afternoon.

	$PM_{0.3}$	$PM_{0.3-0.5}$	$PM_{0.5-1.0}$	$PM_{1.0-2.5}$	$PM_{2.5-5.0}$	$PM_{5.0-10}$
Morning test: Mean (SD.)						
Subject 1	6077 (1013)	1761 (418)	317 (125)	48 (22)	10 (10)	4 (5)
Subject 2	5920 (509)	1399 (212)	238 (60)	27 (9)	3 (3)	1 (0)
Subject 3	5852 (639)	1711 (146)	322 (70)	50 (28)	8 (9)	4 (5)
Subject 5	9671 (1291)	2532 (327)	476 (110)	73 (19)	11 (7)	4 (2)
Subject 6	5992 (1007)	1512 (305)	279 (87)	39 (18)	3 (5)	1 (2)
F (*p*) values*	**31.6 (<0.001)**	**22.3 (<0.001)**	**9.3 (<0.001)**	**7.0 (<0.001)**	**2.9 (0.031)**	2.3 (0.070)
Afternoon test: Mean (SD.)						
Subject 1	5568 (657)	1431 (133)	284 (84)	45 (33)	6 (13)	4 (9)
Subject 2	4774 (1409)	1323 (730)	161 (59)	22 (7)	1 (3)	1 (2)
Subject 3	5663 (584)	1524 (310)	295 (142)	46 (40)	9 (14)	4 (6)
Subject 4	5175 (980)	1284 (302)	216 (76)	41 (14)	5 (4)	3 (2)
Subject 5	5552 (425)	1417 (208)	262 (53)	30 (16)	4 (4)	2 (3)
F (*p*) values*	1.8 (0.154)	0.6 (0.682)	**3.9 (0.009)**	1.7 (0.176)	0.9 (0.480)	0.6 (0.670)

Note: *The results were obtained from one-way ANOVA, *p*-values less than 0.05 are marked in bold.

Table 4. Differences of the number of particles between the situations with and without subjects.

	$PM_{0.3}$	$PM_{0.3-0.5}$	$PM_{0.5-1.0}$	$PM_{1.0-2.5}$	$PM_{2.5-5.0}$	$PM_{5.0-10}$
Morning test: Difference (p)						
Subject 1	**2001.1 (<0.001)**	**707.8 (<0.001)**	**144.2 (0.002)**	**27.0 (<0.001)**	**7.4 (0.006)**	2.9 (0.093)
Subject 2	**1844.8 (0.003)**	**345.4 (0.043)**	65.4 (0.074)	6.8 (0.404)	0.3 (0.859)	0.0 (1.000)
Subject 3	**1776.3 (0.007)**	**657.5 (<0.001)**	**149.4 (<0.001)**	**29.4 (0.007)**	**5.5 (0.021)**	**3.4 (0.047)**
Subject 5	**5595.8 (<0.001)**	**1478.1 (<0.001)**	**303.8 (<0.001)**	**52.4 (<0.001)**	**8.3 (0.004)**	**2.9 (0.004)**
Subject 6	**1916.7 (0.008)**	**458.7 (0.023)**	**106.2 (0.029)**	**17.9 (0.003)**	0.0 (1.000)	0.2 (0.726)
Afternoon test: Difference (p)						
Subject 1	**2047.1 (<0.001)**	**461.3 (<0.001)**	**123.1 (0.006)**	**23.6 (0.002)**	2.4 (0.300)	1.3 (0.369)
Subject 2	**1253.1 (0.014)**	353.4 (0.111)	−0.1 (0.997)	1.0 (0.877)	−2.5 (0.118)	−1.4 (0.138)
Subject 3	**2142.1 (<0.001)**	**554.5 (<0.001)**	**133.5 (0.018)**	**24.8 (0.007)**	4.9 (0.073)	**1.5 (0.030)**
Subject 4	**1653.3 (0.001)**	**314.4 (0.021)**	55.1 (0.071)	**20.0 (0.035)**	0.7 (0.757)	0.0 (1.000)
Subject 5	**2030.9 (<0.001)**	**448.0 (0.001)**	**100.4 (0.022)**	9.0 (0.177)	0.1 (0.955)	−0.7 (0.363)

Notes: 1. The results were obtained from paired samples t-tests; 2. The numbers shown in the table are the differences in the number of particles measured in the tests with and without subjects; 3. The numbers in the parentheses are the *p*-values, and *p*-values less than 0.05 are marked in bold.

Differences in the number of particles between situations with and without subjects. To check whether the device can detect the number of particles in subjects' breathing zone, a series of paired samples t-tests were conducted to compare the number of particles between situations with and without subjects. As shown in Table 4, in most cases, there were statistically significant differences in the number of particles (especially for the small ones) between situations with and without subjects. For $PM_{0.3}$, significant increases were found in the breathing zones of all subjects, compared with the background situation. For $PM_{0.3-0.5}$, $PM_{0.5-1.0}$ and $PM_{1.0-2.5}$, significant increases were found in most subjects' breathing zones compared with the background. While for $PM_{2.5-5.0}$ and $PM_{5.0-10}$, significant increases were only found in a few subjects' breathing zones (and mainly in the morning).

Number of particles and CO_2 concentration in a classroom setting

Figure 4 illustrates the distribution of particles and CO_2 concentrations in the Experience room for different ventilation regimes. Different colours represent different ventilation regimes, and the diameter of bubbles represents the number of particles or the CO_2 concentration (ppm), in logarithmic scales, measured at each point. The average (and standard deviation) number of particles or the average (and standard deviation) CO_2 concentration was noted next
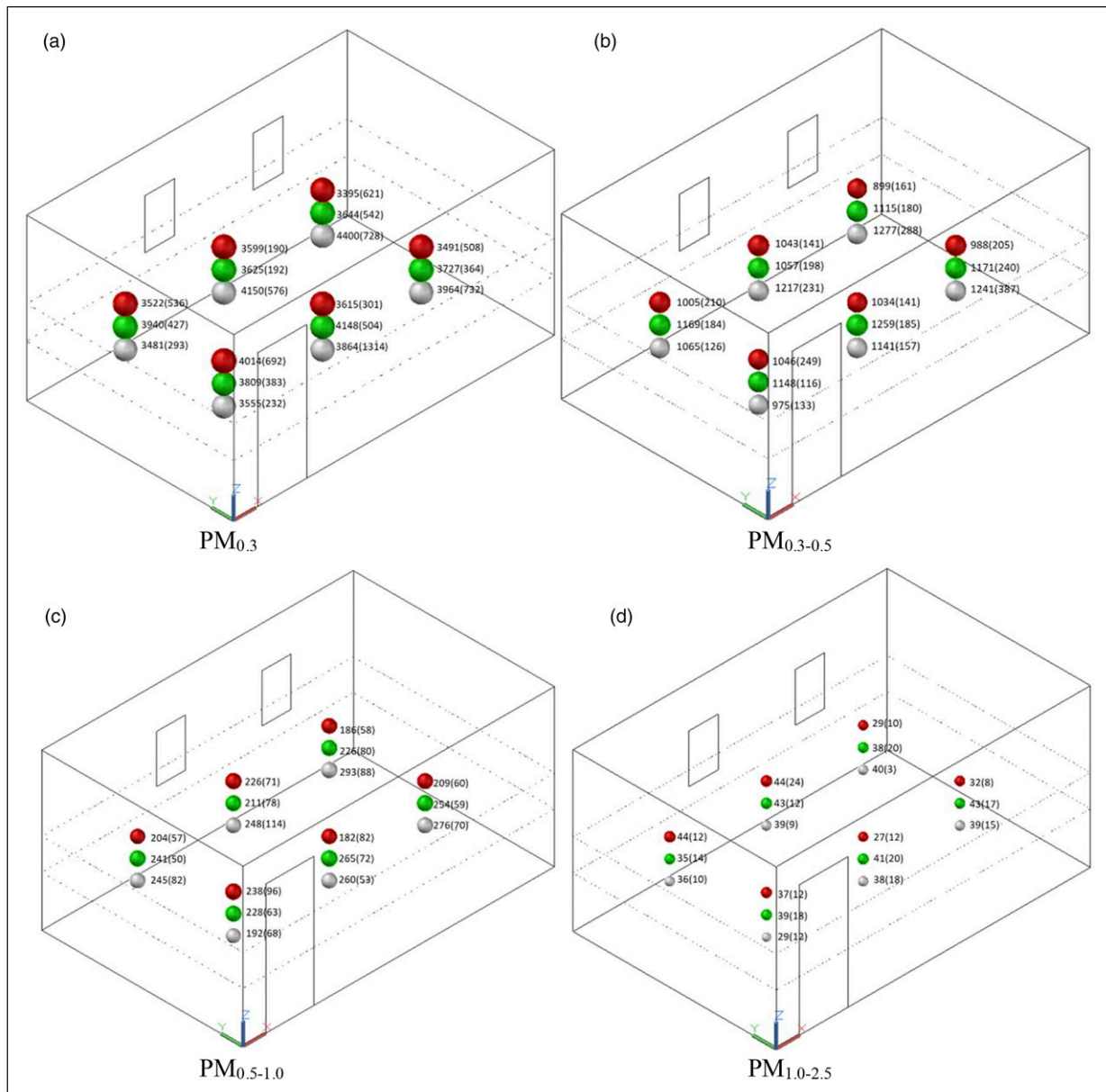
Figure 4. Particles and CO_2 distributions in a classroom setting under different conditions: (a) $PM_{0.3}$; (b) $PM_{0.3-0.5}$; (c) $PM_{0.5-1.0}$; (d) $PM_{1.0-2.5}$; (e) $PM_{2.5-5.0}$; (f) $PM_{5.0-10}$; (g) CO_2. Notes: 1. Red bubbles show the results of 'natural ventilation with subjects'; green bubbles show the results of '600 m^3/h mixing ventilation with subjects'; grey bubbles show the results of '1200 m^3/h mixing ventilation without subjects'. 2. The diameter of bubbles represents the number of particles/CO_2 concentration at logarithmic scales, and in Figures (e) and (f), these numbers were further enlarged five times to make these bubbles visible.

to each bubble. As shown in these figures, the numbers of particles under the condition '1200 m^3/h mixing ventilation without subjects' were similar as the result measured in the test chamber without subjects. However, when there were subjects present, the number of particles measured in the Experience room was larger than the number of particles measured in the box in the test chamber. Moreover, the differences were much more obvious for the smaller

particles ($PM_{0.3}$ and $PM_{0.3-0.5}$). For example, the number of $PM_{0.3}$ was around 3600 in the Experience room, while it was around 2000 in the box in the test chamber.

The normality test results were similar as the previous test, namely, most parameters were normally distributed. Therefore, paired samples t-tests and one-way ANOVA test results were valid. Results obtained from one-way ANOVA tests (i.e. F values and *p* values) are shown in Table 5.
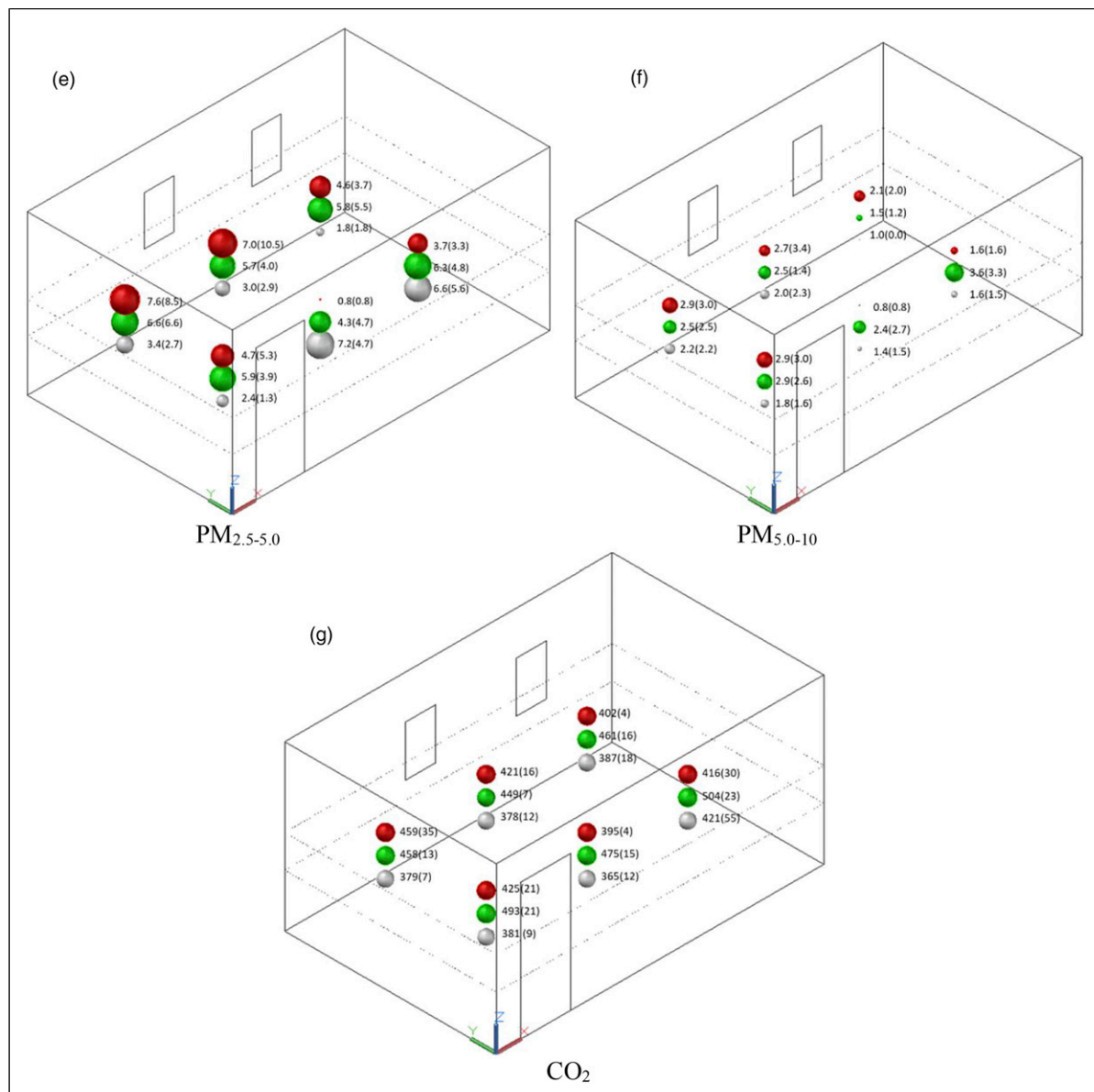
Figure 4. Continued.

Table 5. Results obtained from one-way ANOVA to show the difference of the number of particles between six locations for different ventilation regimes.

	600 m³/h mixing ventilation with subjects	1200 m³/h mixing ventilation without subjects	Natural ventilation with subjects
$PM_{0.3}$	2.3 (0.058)	1.1 (0.374)	1.8 (0.127)
$PM_{0.3-0.5}$	1.3 (0.284)	1.2 (0.357)	0.9 (0.509)
$PM_{0.5-1.0}$	0.9 (0.520)	0.9 (0.493)	0.9 (0.471)
$PM_{1.0-2.5}$	0.4 (0.874)	0.6 (0.709)	**2.7 (0.030)**
$PM_{2.5-5.0}$	0.3 (0.937)	2.1 (0.103)	1.5 (0.198)
$PM_{5.0-10}$	0.8 (0.543)	0.3 (0.896)	1.2 (0.337)

Note: The numbers shown in the table are F-values (*p*-values), and *p*-values less than 0.05 are marked in bold.

Table 6. Results of pairwise comparison between the three tested conditions.

	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6	Overall
'1200 m^3/h mixing ventilation without subjects' V.S. '600 m^3/h mixing ventilation with subjects'							
PM$_{0.3}$	−216.2 (0.559)	352.4 (0.581)	564.4 (0.368)	**1267.8 (0.026)**	628.4 (0.087)	322.6 (0.151)	**486.6 (0.009)**
PM$_{0.3–0.5}$	−76.8 (0.627)	162.6 (0.250)	302.8 (0.284)	**356.2 (0.023)**	**202.4 (0.037)**	211.4 (0.187)	**193.1 (0.003)**
PM$_{0.5–1.0}$	−60.2 (0.298)	69.2 (0.353)	**83.0 (0.016)**	98.2 (0.152)	27.0 (0.381)	69.4 (0.234)	**47.8 (0.025)**
PM$_{1.0–2.5}$	−9.4 (0.185)	10.4 (0.450)	9.2 (0.430)	13.8 (0.057)	−1.0 (0.922)	−2.8 (0.614)	3.4 (0.351)
PM$_{2.5–5.0}$	−2.0 (0.473)	**6.6 (0.040)**	2.0 (0.628)	−2.8 (0.115)	−1.2 (0.516)	−0.8 (0.778)	0.3 (0.788)
PM$_{5.0–10}$	−0.4 (0.825)	0.8 (0.178)	1.0 (0.298)	−0.8 (0.405)	0.4 (0.670)	0.4 (0.828)	0.2 (0.609)
T	**0.1 (<0.001)**	**−0.1 (<0.001)**	**−0.2 (<0.001)**	**−0.3 (<0.001)**	**−0.4 (<0.001)**	**−0.5 (<0.001)**	**−0.2 (<0.001)**
RH	**−0.7 (<0.001)**	**−0.5 (<0.001)**	**−0.4 (0.011)**	**−0.5 (<0.001)**	**−1.5 (<0.001)**	**−1.9 (<0.001)**	**−0.9 (<0.001)**
CO$_2$	**−34.6 (<0.001)**	**−32.8 (0.003)**	24.4 (0.427)	−15.6 (0.131)	**−36.6 (0.029)**	**−88.4 (0.004)**	**−30.6 (0.001)**
'Natural ventilation with subjects' V.S. '600 m^3/h mixing ventilation with subjects'							
PM$_{0.3}$	−205.4 (0.414)	**532.6 (0.017)**	235.9 (0.112)	249.1 (0.207)	25.5 (0.764)	417.8 (0.056)	**209.3 (0.008)**
PM$_{0.3–0.5}$	101.4 (0.145)	**225.0 (0.041)**	**182.7 (0.032)**	**215.8 (0.007)**	13.2 (0.842)	164.4 (0.087)	**150.4 (<0.001)**
PM$_{0.5–1.0}$	−9.9 (0.810)	83.0 (0.060)	44.9 (0.148)	40.7 (0.237)	−14.7 (0.599)	37.6 (0.164)	**30.3 (0.027)**
PM$_{1.0–2.5}$	1.8 (0.796)	14.2 (0.113)	11.6 (0.130)	8.9 (0.156)	−0.2 (0.980)	−8.7 (0.151)	4.6 (0.114)
PM$_{2.5–5.0}$	1.2 (0.376)	**3.5 (0.048)**	2.6 (0.293)	1.2 (0.602)	−1.3 (0.604)	−1.0 (0.755)	1.0 (0.256)
PM$_{5.0–10}$	0.0 (1.000)	1.6 (0.078)	2.0 (0.128)	−0.6 (0.475)	−0.2 (0.878)	−0.4 (0.631)	0.4 (0.332)
T	**0.2 (<0.001)**	**−0.1 (<0.001)**	**−0.1 (<0.001)**	**−0.1 (<0.001)**	**−0.1 (<0.001)**	**−0.1 (<0.001)**	**0.05 (0.017)**
RH	**2.2 (<0.001)**	**1.5 (<0.001)**	**1.0 (<0.001)**	**0.3 (<0.001)**	**−0.5 (0.018)**	**−1.4 (<0.001)**	**0.5 (0.002)**
CO$_2$	**68.4 (<0.001)**	**80.2 (<0.001)**	**88.0 (<0.001)**	**58.8 (<0.001)**	**28.0 (0.001)**	−0.9 (0.950)	**53.8 (<0.001)**
'1200 m^3/h mixing ventilation without subjects' V.S. 'natural ventilation with subjects'							
PM$_{0.3}$	−269.8 (0.247)	−358.6 (0.509)	319.6 (0.494)	821.2 (0.144)	461.2 (0.204)	−380.6 (0.324)	98.8 (0.556)
PM$_{0.3–0.5}$	−166.4 (0.065)	−156.6 (0.094)	61.6 (0.843)	228.8 (0.318)	234.2 (0.168)	−59.0 (0.436)	23.8 (0.728)
PM$_{0.5–1.0}$	−32.2 (0.379)	16.2 (0.761)	4.8 (0.941)	53.0 (0.222)	60.2 (0.409)	14.4 (0.715)	19.4 (0.315)
PM$_{1.0–2.5}$	−0.2 (0.977)	3.8 (0.759)	−2.2 (0.666)	12.2 (0.221)	−4.2 (0.515)	2.6 (0.715)	2.0 (0.510)
PM$_{2.5–5.0}$	−2.4 (0.215)	3.8 (0.241)	2.2 (0.224)	−3.4 (0.142)	**−2.6 (0.041)**	−2.6 (0.386)	−0.8 (0.364)
PM$_{5.0–10}$	−0.2 (0.838)	0.4 (0.477)	−0.2 (0.815)	−0.6 (0.070)	−0.4 (0.757)	0.8 (0.541)	0.0 (0.923)
T	0.0 (0.741)	0.0 (0.541)	**−0.1 (0.001)**	**−0.2 (<0.001)**	**−0.3 (<0.001)**	**−0.3 (<0.001)**	**−0.2 (<0.001)**
RH	**−2.7 (<0.001)**	**−1.9 (<0.001)**	**−1.3 (<0.001)**	**−0.7 (<0.001)**	**−0.5 (0.001)**	**−0.5 (0.001)**	**−1.3 (<0.001)**
CO$_2$	**−100.2 (<0.001)**	**−100.2 (<0.001)**	**−68.0 (0.043)**	**−63.4 (0.001)**	**−68.6 (0.001)**	**−71.0 (<0.001)**	**−78.6 (<0.001)**

Notes: 1. The results were obtained from paired samples t-tests; 2. The numbers shown in the table are the differences in the parameters measured in the two tested conditions; 3. The numbers in the parentheses are the *p*-values, and *p*-values less than 0.05 are marked in bold.

Almost no significant difference in the number of particles was found between the six locations (except for $PM_{2.5}$ under natural ventilation) which indicated an even distribution of all the particles in the room for all ventilation regimes.

Comparison of the number of particles and CO_2 concentration between different conditions. Table 6 shows the results obtained from the paired samples t-tests. In most cases, there were significant differences in T, RH and CO_2 concentrations between any two conditions at all locations. In general, the comparison results were similar for these three parameters: they were all significantly higher in the conditions with subjects than in the conditions without subjects, and they were also significantly higher in the condition with natural ventilation than in the condition with mechanical ventilation.

The overall comparison results between the conditions '1200 m^3/h mixing ventilation without subjects' and '600 m^3/h mixing ventilation with subjects', show that after the subjects left the room and the ventilation rate was increased, the numbers of particles with diameters less than 1.0 μm (i.e. $PM_{0.3}$, $PM_{0.3-0.5}$ and $PM_{0.5-1.0}$) increased significantly. However, according to the comparison results conducted for the individual locations, the differences were only significant at a few locations. For the comparison between the conditions 'Natural ventilation with subjects' and '600 m^3/h mixing ventilation with subjects', after the ventilation regime was changed from mechanical to natural ventilation, significant increases were also observed in the numbers of $PM_{0.3}$, $PM_{0.3-0.5}$ and $PM_{0.5-1.0}$, and the differences were also only significant at a few locations. While for the comparison between the conditions '1200 m^3/h mixing ventilation without subjects' and 'Natural ventilation with subjects', after the ventilation regime was changed from mechanical to natural ventilation and after the subjects entered the room, no significant changes in the number of particles occurred.

Relationships between the number of particles and CO_2 concentration under different conditions. Table 7 shows the Pearson correlation coefficients between the CO_2

concentration and the number of particles measured in the Experience room. Different results were found for different conditions. For '1200 m^3/h mixing ventilation without subjects', no significant correlations between the CO_2 concentration and the number of particles were observed. For '600 m^3/h mixing ventilation with subjects', as shown in Table 7, there was a significant positive correlation between the CO_2 concentration and the number of $PM_{5.0-10}$. As shown in Figure 3, at the positions where the CO_2 concentrations were high, the numbers of $PM_{5.0-10}$ were also high. For 'Natural ventilation with subjects', the numbers of $PM_{2.5-5.0}$ and $PM_{5.0-10}$ were found to be significantly related to the CO_2 concentration. The Pearson correlation coefficient was larger with a larger particle size.

Discussion

Number of particles measured in different people's breathing zone

The comparisons presented in Table 3 showed almost no differences in the number of particles between different subjects' breathing zones, except for one subject in the morning test. More particles were measured in this subject's breathing zone than for the other subjects in the morning, while such difference was not observed in the afternoon. This might have been caused by the breakfast this subject ate before the experiment which was a hot meal and contained several kinds of herbs and spices. Since all the subjects had the same lunch, there was no difference caused by the food in the afternoon.

Although differences in the number of exhaled particles between different people have been found before, those differences were explained by the different collecting and measuring methods[20,43] or health status.[44] Since only one measurement method and one device were used in this study, all the subjects were healthy and their body mass indexes were similar, therefore, it makes sense that almost no significant differences in the number of exhaled particles were measured between these subjects in the current study.

Table 7. Relationships between the number of particles and CO_2 concentration.

	$PM_{0.3}$	$PM_{0.3-0.5}$	$PM_{0.5-1.0}$	$PM_{1.0-2.5}$	$PM_{2.5-5.0}$	$PM_{5.0-10}$
1200 m^3/h mixing ventilation without subjects	0.23 (0.227)	0.42 (0.020)	0.23 (0.219)	0.14 (0.451)	0.21 (0.264)	0.08 (0.688)
600 m^3/h mixing ventilation with subjects	0.10 (0.451)	0.19 (0.151)	−0.03 (0.833)	0.21 (0.109)	0.09 (0.513)	**0.29 (0.023)**
Natural ventilation with subjects	0.11 (0.416)	0.01 (0.955)	0.06 (0.672)	0.22 (0.093)	**0.32 (0.013)**	**0.42 (0.001)**

Notes: 1. The results were obtained from Pearson correlation analyses; 2. The numbers shown in the table are the Pearson correlation coefficients; 3. The numbers in the parentheses are the *p*-values, and *p*-values less than 0.05 are marked in bold.

According to the outdoor measurement results, the concentration of outdoor fine particles was more than twice as much as those in the subjects' breathing zones, which also confirmed the findings of previous studies that outdoor particles are the main source of indoor particles.[45–47] On the contrary, the main part of the indoor CO_2 concentration was produced by humans through breathing (the CO_2 concentration in the exhaled breath can be 100 times higher than the outdoor level[48]) and therefore, it can be used to decide the maximum room occupancy.[49] The different exhalation/outdoor concentration ratios between particles and CO_2 make it difficult to use the CO_2 concentration as a proxy for the number of exhaled particles.

Number of particles and CO_2 concentration in a classroom setting

As shown in Table 6, compared with '600 m^3/h mixing ventilation with subjects', the number of small particles (particles with diameters less than 1 μm) was increased significantly in the condition of '1200 m^3/h mixing ventilation without subjects'. This might be explained by the fact that particles came through the ventilation ducts, so, the higher the ventilation rate, the more particles were detected. The ventilation system used in the SenseLab has F7 filters which can filter more than 65% of PM$_{2.5}$, but it cannot effectively filter out particles smaller than 1 μm. Regarding the comparison between conditions '600 m^3/h mixing ventilation with subjects' and 'Natural ventilation with subjects' (in which the subjects were the same, the only difference was that the ventilation changed from mechanical to natural), numbers of small particles also increased significantly. This might indicate that the numbers of small particles directly coming through windows were larger than those coming through ventilation ducts when the ventilation rate was relatively low (600 m^3/h). For the comparison between '1200 m^3/h mixing ventilation without subjects' and 'Natural ventilation with subjects', no significant difference between the particle numbers was found. One possible explanation is that the number of particles directly coming from outside (through the windows) plus those generated by subjects equals the numbers of particles coming through the ventilation ducts when the ventilation rate was relatively high (1200 m^3/h). All of these characteristics of indoor particles were quite different from CO_2 which was significantly lower in the condition without subjects than in the condition with subjects, and also significantly lower with mechanical ventilation than with natural ventilation. These results indicate that most indoor particles measured in this study originated from outdoors (either through windows or through ventilation ducts). Therefore, the higher the ventilation rate the more particles (especially the small ones) were monitored in the room, which was the reverse for the CO_2 concentration.

Relationships between the numbers of particles and CO_2 concentrations in a classroom setting

In terms of the relationships between the number of particles and CO_2 concentration, only the coarse particles (particles with a diameter larger than 2.5 μm and smaller than 10 μm) were found to be significantly correlated with the CO_2 concentration when there were subjects inside the room. Similar results were also reported by Lazović et al.[50] and Fromme et al.[51] However, these two studies did not consider smaller particles, and no significant relationships were found between the CO_2 concentration and particles with a diameter less than 2.5 μm in the current study. A possible explanation could be that the activities people performed increased both the indoor CO_2 concentration and the number of coarse particles.[52,53] Nevertheless, the fine particles (with a diameter less than 2.5 μm) deserve more attention since they have been shown to be the major component of respiratory particles and possibly the main carriers of SARS-CoV-2.[18] The not significant relationship between the number of fine particles and the CO_2 concentration observed in this study could be explained by the fact that the main source of indoor particles is outdoor air, while the main source of CO_2 indoors is the occupant.

Given the outcome that (1) most measured indoor particles came from outdoors and exhaled particles only presented a very small portion of them, while exhaled CO_2 presented a large portion of indoor CO_2; (2) the impact of the ventilation regime on indoor CO_2 concentration was different compared to the impact on the number of indoor particles; and (3) the relationship between the number of most indoor particles and CO_2 concentration was not significant, it is impossible to conclude that the indoor measured CO_2 concentration is a good proxy for the number of exhaled indoor particles.

Limitations and future studies

The findings of this study have to be seen in the light of some limitations. The first one is the number of participants which was lower than the occupancy in real classrooms. Because this study was conducted during the COVID-19 pandemic, to ensure the safety of all the participants, more than 1.5 m social distance was kept between each of them. In future studies, more participants should be involved to test the real situation in classrooms. The second limitation is the filtration of outdoor air. Because of the limitation of the filters used in the HVAC system of the Experience room, the smaller particles were not filtered out from the outdoor air,

which made it difficult to study the characteristics of the exhaled particles, and hence, impossible to draw a definite conclusion on the relationship between CO_2 concentrations and exhaled particle numbers. To better understand the distribution of exhaled particles, future studies should be conducted in a clean environment without any other particles, or at least filter out outdoor particles as much as possible.

Conclusion and recommendation

To verify whether the CO_2 concentration can be used as a proxy for the number of exhaled aerosols in a classroom setting, two different experiments were performed: the measurement of (1) the number of particles with different sizes and CO_2 concentrations in a person's breathing zone and (2) the distribution of particles with different sizes and CO_2 in a classroom setting under different ventilation regimes. The results showed that (1) contrary to CO_2, the main source of indoor particles came from outdoors and not from occupants; (2) the impact of ventilation regimes on indoor particle numbers was different compared to the impact on CO_2 concentration; and (3) almost no significant relationship between the number of indoor particles and CO_2 concentration was observed. Based on these results, this study could therefore not conclude that the CO_2 concentration in a classroom can be used as a proxy for the number of exhaled particles by the occupants.

To study the distribution of exhaled particles, more research with alternative methods, such as visualization techniques in combination or not with tracers, such as soap bubbles or coloured mist produced by a mannikin head, is needed. Although the particle counter used in this study has proven to be a reliable device to measure aerosol concentrations, it cannot distinguish the exhaled particles from the particles originating from outdoors, being the main source of indoor particles. Therefore, a particle counter can in fact not accurately measure the exhaled number of particles, unless all the particles coming from outdoors (or other sources than breath) can be filtered out.

Author contributions

Dadi Zhang: conceptualization; methodology; investigation; data analysis; writing-original draft preparation; writing-review and editing. Philomena Bluyssen: conceptualization; methodology; supervision; writing-original draft preparation; writing-review and editing.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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