Indoor air quality analysis in naturally ventilated university training laboratories: a health risk assessment

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Abstract
The indoor air quality (IAQ) of eleven naturally ventilated training laboratories was analysed to evaluate the health risk to occupants. IAQ evaluation included analysis of physical (temperature (T) and relative humidity (RH)), chemical (CO2, CO, O3, total volatile organic compounds (TVOC), and particulate matter (PM)) and microbiological (fungi and bacteria) pollutants. Monitoring was carried out in labs used for teaching different academic disciplines (biology, chemical, ecology, and computers) during two periods of the academic year. Ventilation rates (VR), air change per hour (ACH) in every lab, and the hazard quotients for each of the chemical pollutants and the accumulated (HQ and HI) were calculated. Environmental comfort was not fully satisfactory considering the RH and CO2 values, especially during hours with higher occupancy. Coarse particles and bacteria were generated indoor related to human activity. At chemical and biological laboratories, TVOC concentrations were sometimes above the recommended value, and all the labs presented VR below the European guideline’s recommendations. Results from this study show natural ventilation is not enough to get an adequate IAQ, although no significant non-carcinogenic risk was estimated. However, installation of complementary ventilation systems would be advisable to avoid health risk by acute short-term exposure.

Keywords Indoor air quality · Training laboratories · Ventilation rate · Chemical pollutants · Microorganisms · Health risk

Introduction
People in developed countries spend over 90% of their time in indoor environments (Billionnet et al. 2011), and therefore, the maintenance of adequate indoor air quality (IAQ) is an important health concern. IAQ can be affected by several factors, such as the closure of natural openings in buildings for energy-saving purposes, which results in poor air exchange, or the use of new materials and/or cleaning products, which causes an increase in pollutant concentrations (Stathopoulou et al. 2008). The most common manifestations of poor IAQ are some non-specific symptoms such as headache, eye or nasal irritation, rashes or itching, malaise, or concentration difficulties, which generally cannot be attributed to specific causes, and their occurrence is often described as sick building syndrome (SBS) (Gupta et al. 2007). Moreover, long-term exposure to indoor pollutants can be responsible for more severe health problems, including respiratory and cardiovascular pathologies, sensory disturbances, reproductive problems, allergic diseases, or even cancer (Tsakas et al. 2011). The World Health Organization (WHO) (WHO 2010) claimed that indoor exposure to air pollutants can cause very significant damage to health, globally.

IAQ is usually determined using different criteria based on the concentrations of a set of chemical pollutants—such as carbon monoxide (CO), ozone (O3), particulate matter (PM), and different volatile organic compounds (VOCs)—together with comfort variables like carbon dioxide (CO2) concentration, temperature (T), and relative humidity (RH). It is generally accepted that the levels of these parameters depend on outdoor air pollution, the type of indoor activity, physical building characteristics, or the air exchange rate (Frontczak et al. 2012; Schweiker et al. 2012). It has also been demonstrated that indoor air pollution levels can often exceed outdoor levels (Assimakopoulos and Helmis 2004; Halios et al. 2005; Nguyen et al. 2014; Stathopoulou et al. 2008).
In addition to chemical pollutants, in recent years, the presence of bioaerosols has been considered in IAQ analysis given their probable impact on human health. Bioaerosols are airborne particles containing living organisms that can be generated from various natural and anthropogenic sources and that, therefore, contain pathogenic and/or non-pathogenic dead or alive microorganisms (Kim et al. 2018). Due to their small size and mass, they are easily transported and persist in the air for long periods (Brown and Hovmöller 2002). The existence of bioaerosols in the air of indoor environments is clearly inevitable; however, depending on their concentration and microbial composition, they can affect the IAQ, thus becoming a specific disease risk factor. The quantity and composition of the microbiota existing in the air of indoor environments are mainly dependent on the microclimate, especially T and RH, as well as the presence of some chemical contaminants and suspended PM (Dedesko and Siegel 2015). Indeed, RH is one of the factors with the most influence on the survival of microorganisms in the air. While some types of viruses require extreme humidity levels to live, others, including certain bacteria, can survive in the form of bioaerosols in environments with a limited range of humidity. Furthermore, the survival of microorganisms and house dust found on the surfaces would increase at an RH above 60% and can cause respiratory problems (Widya et al. 2019).

Ventilation is a factor that plays a crucial role in achieving adequate air quality and a comfortable and healthy indoor environment (Mendell et al. 2013; Rackes and Waring 2014). It promotes the exchange of outdoor air, removing or diluting indoor chemical and biological pollutants, with the air exchange rate being a critical parameter for assessing and interpreting IAQ. CO₂ concentration is an indicative parameter of IAQ, used to determine the ventilation rate (VR) (Bulińska et al. 2014), so if the CO₂ levels exceed 1,000 ppm, this indicates a lack of adequate ventilation (ANSI/ASHRAE 2019), and occupants commonly complain about headaches, nose, and throat ailments, tiredness, lack of concentration, and fatigue (Seppälä et al. 1999; Siskos et al. 2001). VRs below 10 L/s × person can enhance the appearance of SBS, while values higher than 20 L/s × person have been reported to significantly decrease the appearance of the associated symptoms (Seppälä et al. 1999). However, low CO₂ levels do not necessarily indicate good IAQ, and therefore, it is also necessary to perform control measurements for other air pollutants.

Universities are higher education learning institutions and carry out the educational work to students. Many different facilities (classrooms, practice labs, libraries, gyms, dining rooms, etc.) are used, which vary according to the curricular planning. From an IAQ point of view, training labs present a special interest because in them, one can find specific pollutants depending on their usage (Park et al. 2014). These laboratories often present a closed, crowded environment, with many pieces of large electronic equipment and the regular use of toxic substances during experimental processes, all of which constitute environmental pollution sources. Students and teachers spend a significant proportion of their training time in these spaces, and therefore, the maintenance of adequate IAQ is essential for the enhanced performances of students and staff members since poor air quality can affect their health and productivity (Jin et al. 2018).

Some authors (Annesi-Maesano et al. 2013; Hulin et al. 2010; Mendell et al. 2013) have reported that poor IAQ in university facilities can lead to reduced comfort for students, which would influence their attendance and learning performance. Moreover, in the case of chemical and biology laboratories, the users (staff, instructors, assistants, and students) would be exposed to chemical and/or microbiological contaminants, which could cause acute and chronic health effects (Widya et al. 2019). Some studies related to IAQ in research and teaching university laboratories have been reported previously (Rumchev et al. 2003; Valavanidis and Vatista 2006; Ugranli et al. 2015; Saad et al. 2016; Telejko 2017; Jin et al. 2018; Kwong et al. 2019; Widya et al. 2019; Mishra et al. 2020) but in most of them, only chemical pollutants and comfort parameters are evaluated. Some of the most recent studies reported a focus on the assessment of only one specific pollutant (Feng et al. 2020; Jin et al. 2018; Meng et al. 2020).

Hazrin et al. (2015) carried out a combined study analysing chemical, physical, and microbiological parameters in research laboratories. More recently, Idris et al. (2020) reported results from a combined study performed in two university labs used by students both for learning and research activities. However, none of them included the VR of spaces being considered. Therefore, the purpose of this work was to carry out a multidisciplinary study of the IAQ in different teaching laboratories at the Environmental Science and Biochemistry Faculty of Castilla-La Mancha University (Toledo, Spain), combining physical, chemical, and microbiological analysis with the assessment of ventilation efficiency.

Laboratories used for teaching different academic disciplines (biology, chemistry, ecology, and computers) were sampled to assess the pollutants to which students would be exposed. CO, CO₂, O₃, total volatile organic compounds (TVOC), particle number (PN) of different size (PNₐ, PNₐ₁, and PNₐ₋₁), as well as the values of T and RH, were measured and counts of fungi and bacteria were carried out using two sampling methods (impaction and gravity). In addition, measurements of the same parameters in the outdoor air were carried out to elucidate potential airborne contamination sources.

The standard requirements for thermal comfort and ventilation in training laboratories are reported in RD (2007) and
ANSI/ASHRAE (2019), respectively. It is important to highlight that, in Spain, there is no law regulating the maximum reference concentration of chemical pollutants and microorganisms in indoor air, although there is a normative that is not compulsory to obey (UNE 2014) establishing the limit values and comfort criteria for these parameters in indoor environments. Therefore, the results for all the measured parameters were compared with those of the recommendations of the Spanish normative. A health risk assessment was applied to assess the non-carcinogenic risk to laboratory users from exposure to the chemical pollutants. The correlations among all the studied parameters were also analysed.

**Methods**

**Location and sampling**

This study was carried out in 11 laboratories used for the practical teaching of different academic disciplines, located at different buildings in the Fábrica de Armas Campus of the University of Castilla-La Mancha (Toledo, Spain) (Fig. 1). These buildings are close to the Tagus riverbanks, in a pedestrian area surrounded by abundant riparian vegetation and soil with a gravel surface layer. This area presents no significant direct vehicle emissions, although it is surrounded by neighbourhoods with some road traffic.

The studied laboratories were chosen based on their different activity characteristics: two of them were used for data analysis and equipped with computers (labs labelled PC1 and PC2), and the remaining were used, with specific equipment, for practical teaching in ecology (lab labelled ECO), chemistry and chemical engineering (labs labelled CHE1–CHE4), and biology (labs labelled BIO1–BIO4). All the studied laboratories are located on the first floor of four different buildings, which comprise more laboratories used for teaching similar subjects. The interior design of each lab depends on its use, but every lab has a door that opens to a main lobby and several windows proportional to size. Figure 2 shows the layout plans and images of the sampled labs; Table 1 contains detailed information about their sizes and specific uses.

The labs have a central heating system, but they are not equipped with mechanical ventilation systems, and only natural ventilation takes place. During the teaching period, ventilation occurs through a small grille located on one of the laboratory walls that is connected to the outside. At the end of this period, when the students leave the labs, the doors

![Fig. 1 Situation of sampled laboratories at the University of Castilla-La Mancha (UCLM) Campus in Toledo (Spain). (CHE: Chemical laboratories; BIO: Biological laboratories; PC: Computer laboratories; ECO: Ecology laboratory)](image-url)
and/or windows are opened for a short period to increase ventilation. The chemistry and biology laboratories have fume ventilation hoods, which operate at full speed while practical lessons are taking place, to prevent the exposure of personnel and students to the fumes of volatile chemicals. During monitoring, the central heating was turned on, and the door and windows were closed to avoid potential interferences from outdoor air and heat loss.

The study was carried out in two periods of the academic course 2018–2019: the first period was October–December 2018, and the second period was February–March 2019. A total of 60 days of measurements were conducted, and each laboratory was monitored between 17 and 34 times each academic year.

The laboratories were usually occupied from Monday to Friday, from 10:00 a.m. to 7:30 p.m., with a lunch break from 1:00 p.m. to 4:00 p.m. They were studied both when the number of students inside reached maximum occupancy and during the lunch break to monitor the VR. In addition, monitoring of the unoccupied labs was taken during periods without practical teaching (January 2019) to acquire a baseline reference for non-activity conditions. Information related to the occupancy and the use of chemical solvents, biological materials, or Bunsen burners was recorded.

Simultaneously with the indoor monitoring of the labs, outdoor air was also studied. For this purpose, the measuring equipment was placed approximately 4 m from the buildings of the labs, in a natural unpaved landscape of the university campus, where traffic is very restricted.

Physical and chemical monitoring

Concentrations of the following chemical pollutants were measured: CO₂, CO, PN, TVOCs, and O₃; T and RH were also monitored. Physical and chemical sampling was carried out using instruments enabling real-time measurement.

The indications of the WHO (2020), related to the procedure of sampling and analysis of chemical pollutants in indoor spaces, were followed. For indoor air monitoring, the measuring instruments were positioned on a workbench situated in the centre of each lab at a height of 1.5 m above floor level at the laboratories where students worked standing up (Lab-CHEs, Lab-BIOs, and Lab-ECO) and at 1 m in the laboratories where students sat (Lab-PCS) (see red points in Fig. 2). For outdoor air, they were on a portable table at 1 m height from the floor. The outdoor weather data during the monitoring period are shown in the Supplementary Information (SI) (Fig. S1) (Cornes et al. 2018).

The direct reading instruments used, and their respective accuracies are summarised in Table 2. Each monitoring day, the data from all these instruments were recorded every five minutes for approximately 1 h, calculating the data average.
| Activity       | Chemistry and Chemical Engineering | Biology       | Computer | Ecology | Outdoor |
|----------------|-----------------------------------|---------------|----------|---------|---------|
| Laboratory     | CHE.1                             | BIO.1         | PC.1     | ECO.1   |         |
| Dimensions (m³) | 445                               | 506           | 303      | 328     | 330     |
| Ventilation rate (VR) (l/s person) | 2.2±0.7                       | 5.6±0.8       | 3.4±0.5  | 2.8±0.5 | 5.9±0.6 |
| Air change per hour (ACH)       | 0.4                               | 0.5           | 0.5      | 0.5     | 0.5     |
| Size/occupancy ratio (m³/person) | 32                               | 23            | 16       | 23      | 26      |
| n*                         | 21                                | 21            | 21       | 46      | 42      |
| T (°C)                     | Mean±SD 21.3±1.2                   | 20.8±1.0      | 20.7±1.9 | 20.3±2.0 | 20.6±2.0 |
| R.H. (%)                   | Mean±SD 38±8                      | 42±7          | 41±10    | 42±8    | 42±10   |
| CO (ppm)                   | Mean±SD 963±242                    | 896±186       | 742±92   | 1049±208 | 876±250  |
| CO₂ (ppm)                  | Mean±SD 2.8±3.0                    | 1.93±4.22     | 2.78±3.81 | 0.75±0.52 | 1.00±1.60 |
| TVOCs (μg/m³)              | Mean±SD 1002±889                   | 1175±1306     | 125±320  | 321±155 | 321±155 |
| O₃ (ppm)                   | Mean±SD 15.0±15.4                  | 24.1±7.9      | 17.5±10.4 | 17.0±8.6 | 16.0±8.6 |
| PN₀.₃ (part/cm³)           | Mean±SD 10.3                      | 17.0±13.5     | 26.4±5.7 | 42.6±5   | 42.8±5  |
| PN₀.₅ (part/cm³)           | Mean±SD 5.0                       | 27.0±15.5     | 37.4±4.4 | 25.7±3.1 | 25.5±3.4 | 25.5±3.4 |
| Bacteria Impaction (CFU/m³) | Mean±SD 681±377                    | 605±277       | 734±442  | 221±307 | 209±357 |

**Table 1** Minimum, maximum and mean values (± standard deviation) for environment comfort indicators, chemical pollutants and indoor airborne microorganisms in the laboratories sampled.
Microbiological sampling

Counts of total culturable bacteria and fungi were performed. For air sampling, both the MAS-100 Eco® microbial air sampler (Millipore), operating according to the impaction method, and the gravity method were used. For the first method, a defined air volume (200 L for indoor samplings and 500 L for outdoor samplings) at a flow rate of 100 L/min, impacted a culture medium in a Petri dish where the airborne microorganisms were retained. In the second method, a Petri dish (diameter 90 mm) containing an adequate culture medium was kept open for one hour to allow the microorganisms to settle. Both the air sampler and the opened Petri dishes were placed in the same place that the remaining instruments used.

The culture media were trypticase soy agar (TSA), supplemented with 100 ppm cycloheximide, and rose bengal agar (RBA), supplemented with 50 ppm chloramphenicol, to count the total airborne bacteria and fungi, respectively. Both media were purchased from Scharlau (Barcelona, Spain). For each sampling method, two plates of each culture medium were used. The plates were aerobically incubated at 30 °C for 3 days and 25 °C for 5 days for bacteria and fungi growth, respectively. The colony forming units (CFU) on the plates were counted, and the mean ± standard deviation was calculated. For the impaction method, the results were expressed as colony forming units per cubic meter of air (CFU/m³) using the positive-hole correction method (Andersen 1958), and as colony forming units per plate per hour (CFU/plate/h) for the gravity method.

Determination of ventilation rates

The VRs were determined by the ‘decay method’ (ASTM 2011, 2012) that fitted the exponential-like decrease in CO₂ concentrations during the unoccupied period. Air change per hour (ACH) can be calculated from measured concentration values at a time, $t$ (Alves et al. 2013):

$$ ACH = \frac{1}{t} \ln \left( \frac{C_0 - C_{out}}{C_i - C_{out}} \right) $$  \hspace{1cm} (1)$$

where $C_0$ is the concentration of CO₂ in the indoor air at time 0, $C_{out}$ is the outdoor concentration of CO₂, and $C_i$ is the indoor concentration of CO₂ at time, $t$. Considering the volume of each laboratory (V) and the occupancy, the air flow rate per person, VR, could be obtained from the following equation:

$$ VR = ACH \times V / n^{*} \text{occupants} $$  \hspace{1cm} (2)$$

(*) number of samplings

(**) mean of the samplings above detection limit

(***) LOD indicates a value below the limit of detection
Thereby, the fraction of laboratories that exceeded the current minimum VR recommendation for educational laboratories (5 L/s × person) (ANSI/ASHRAE, 2019) was determined.

**Health risk assessment**

Health risk assessment for air pollution is an estimation of the expected health impact from exposure to air pollutants (Hassan Bhat et al. 2021). In this study, the non-carcinogenic health risk has been evaluated from the hazard quotient (HQ), which is the ratio of potential exposure to pollutants and its concentration without adverse health effects (Kaewrat et al. 2021):

\[
HQ = \frac{ADD}{RfD}
\]  

(3)

where the average daily dose ADD (mg/kg × day) is the exposure to pollutants by respiratory inhalation (mg/kg × day), and the reference dose (RfD) refers to an estimated level of human daily intake without adverse health effects during a lifetime (mg/kg × day).

The ADD was calculated from Eq. (4) (Kaewrat et al. 2021).

\[
ADD = \frac{CA \times IR \times ET \times EF \times ED}{BW \times AT}
\]  

(4)

where CA is the average concentration for each pollutant (mg/m³), IR is the inhalation rate (m³/hour), ET is the exposure time (hours/day), and EF is the exposure frequency (3).

**Table 2** List of instruments and their characteristics used for experimental measurement

| Instrument model       | Pollutant | Sensor type       | Range          | Accuracy | Resolution |
|------------------------|-----------|-------------------|----------------|----------|------------|
| Testo 315-3 meter CO₂  | NDIR      | 0 – 10000 ppm     | ±5%            | 10 ppm   |
| CO                     | Electrochemical | 0 – 100 ppm     | ±3 ppm         | 0.5 ppm  |
| T                      | Pt-100 RTD | –10 – 60 °C       | ±0.5°C         | 0.1°C    |
| R.H                    | Capacitive | 5 – 95 %          | ±2.5%          | 0.1%     |
| Kanomax Model 3887 PN  | Laser Particle Counter | 0.3, 0.5 and 5 μm | ±5% | ≤15% (for PSL particles near 0.3μm) |
| ToxiRAE Pro PID TVOCs  | Photoinization | 0.1 – 2000 ppm   | ±3%            | 0.1 ppm  |
| AeroQual-SM 70 O₃     | Gas sensitive semiconductor | 0.001 and 0.15 ppm | ±0.005ppm | 0.001 ppm |

**Table 3** Exposure factor of air pollutants in the laboratories

| Exposed Parameters | CO (mg/m³) | O₃ (mg/m³) | Acetone | Hexane | PM |
|--------------------|------------|------------|---------|--------|----|
| CA (mg/m³)ᵃ       | 0.002      | 0.013      | 1.175   | 1.002  | 0.005 |
| IR (m³/hour)ᵇ      | 0.54       |            |         |        |     |
| ET (hour/day)ᶜ     | 4          |            |         |        |     |
| EF (day/year)ᵈ     | 19         | 16         | 15      | 18     | 50  |
| ED (year)ᵉ         | 4          |            |         |        |     |
| BW (kg)           | 65         |            |         |        |     |
| AT (days)          | 1400       |            |         |        |     |
| RfC (mg/m³)ᵇ       | 0.340      | 0.019      | 18.970  | 0.600  | 0.006 |
| ADD (mg/kg day)     | 3.52×10⁻⁶ | 1.99×10⁻⁵ | 1.67×10⁻³| 1.71×10⁻³| 2.48×10⁻⁵|
| RfD (mg/kg day)     | 0.068      | 0.004      | 3.782   | 0.120  | 0.001 |
| HQ                 | 0.00       | 0.01       | 0.00    | 0.01   | 0.02 |

ᵃCA: average concentration for each of the pollutants
ᵇIR: inhalation rate for adult (Kaewrat et al., 2021)
ᶜET: exposure time
ᵈEF: exposure frequency or days per year with exposure at each of the pollutants
ᵉED: 4 years of the degree were considered for exposure duration
ᶠBW: Body weight was 65 kg for adults (Kaewrat et al., 2021)
ᵍAT: the averaging time can be considered equal at ED×365 days/year
ʰRfC for every pollutant was obtained of (https://www.epa.gov/risk/regional-screening-levels-rsls-generic-tables) for hexane and (Silva et al., 2016) for O₃ and PM. For CO and acetone RfC value was not found, thus minimal risk level was used (ATSDR, 2012; 2021)
(days/year). ED is the exposure duration (years), BW is the body weight (kg), and AT is the average time (days).

$RfD$ can be estimated from the corresponding value of the reference concentration (RfC) by Eq. (5).

$$RfD = \frac{RfC \times IR}{BW}$$

Values for parameters included at both equations are shown in Table 3. HQ values less than 0.1 show no hazard exists; HQ values in the range of 0.1–1.0 show a low hazard risk; HQ values in the range of 1.1–10 show a moderate hazard risk, and finally, those over 10 show a high hazard risk (EPA 2007). Additionally, the total non-carcinogenic risk that estimates the risk from exposure to many pollutants at the same time was calculated from the hazard index (HI), which is the sum of HQ for each of the pollutants (Gruszeczka-Kosowska 2018).

**Statistical analysis**

Multiple statistical analysis techniques were used to compare IAQ pollutant concentrations and test for statistically significant differences between the two sampled periods and the use of the labs. First, the normality of all the variables was analysed using the Kolmogorov–Smirnov test. When the data were normally distributed, a $t$-Student test of independent samples was performed to study temporal variability, the non-parametric Mann–Whitney $U$ test in the opposite case. The variations, depending on the use of each laboratory, were also studied via ANOVA for normally distributed variables and the Kruskal–Wallis non-parametric H test when the variables did not comply with the normality. The non-parametric Spearman rank correlation test was used to determine the relationships among the concentrations of chemical pollutants, the microbial counts, and the physical parameters. In all cases, $p$-value < 0.05 was considered significant. Statistical analysis was performed using IBM SPSS Statistics 23.

**Results and discussion**

**Temporal variations**

A total of 11 different laboratories were assessed across two monitoring periods during an academic course. According to the statistical analysis, no significant differences ($p$-value > 0.05) between the measurements of both periods were observed for the studied parameters (see SI, Fig. S2), even though the outdoor meteorological conditions varied during the months sampled (Fig. S1). This fact could be explained by the conditions in which the studies were carried out since, during colder months, heating was turned on and the doors and windows were usually closed. Consequently, external interferences, which could cause variations between measurements obtained at both periods, were minimal. It is important to note that this study could not be expanded to warmer months, when the heating would be off and likely the windows opened, because there was no experimental teaching during those months and the laboratories were unoccupied, so the effect of seasonal variations could not be analysed.

**Influence of occupation and activity on indoor air quality**

Table 1 presents the basic statistics (maximum, minimum, mean, and standard deviation) of all the analysed parameters. Moreover, it describes the activity developed in each laboratory, the size ($m^2$), the occupancy during sampling, and the ratio size/occupancy. The results obtained for all the parameters measured are discussed below.

**Indoor environment comfort indicators**

The average values for indoor environmental comfort indicators—$T$, RH, and CO$_2$ concentration—are shown in Table 1. As expected, the averaged $T$ was extremely similar among the labs, with a mean value of 20.7 ± 0.5 °C (Fig. 3a) since the heating was turned on during sampling. Lab-PC.2 was the coldest setting, with an average temperature of 19.3 ± 2.1 °C, which was very surprising because the computers were on practically all day and, as reported by Telejko (2017), are an additional heat source. The reason could be related to a heating malfunction. Nevertheless, even with this problem, the differences in temperatures among the laboratories were not statistically significant ($p$-value > 0.05). Similar behaviour was found for the RH, being an average at the 11 monitoring laboratories of 42 ± 2%, with the lowest average value in Lab-CHE.1 (38 ± 8%) and the highest one in Lab-BIO.4 (45 ± 9%) (Fig. 3a).

The environmental comfort range is a variable that depends on local geography and climate (Seppänen and Fisk 2006). The established values in the Spanish legislation for indoor environments during the autumn and winter seasons are 21–23 °C for $T$ and 40–50% for RH (RD 2007). Table 4 shows the percentages of the measurements out of the recommended ranges in each laboratory. The highest deviations from the compulsory temperature range were observed for computer labs. However, for the RH, 36% of the total measurements were outside the recommended range, with most of them below the recommended limit and just five over it.
Fig. 3  Indoor environmental comfort indicators, chemical pollutant concentrations and airborne microorganism counts in the laboratories sampled
During the colder months, the heating systems dry outdoor air, so the RH value for indoor air drops below the recommended value. Low RH can cause SBS symptoms such as eye irritation, throat irritation, and coughing, and it increases the susceptibility to infectious diseases and asthma (Ratodi et al. 2017).

Humans are the main source of CO₂ within indoor spaces because of respiration (Yau et al. 2012). Figure 3b shows the CO₂ concentration and the size/occupancy ratio in each laboratory. The overall mean CO₂ concentration in the 11 studied laboratories was 940 ± 222 ppm when they were occupied, with values ranging between 450 ppm and 1,710 ppm. In general, the CO₂ concentration was correlated with the occupancy in the laboratory during monitoring and its size. The biology laboratories (Labs-BIO) showed the highest values for CO₂ concentration even when their occupancy was equal to or less than that of the chemistry laboratories (Labs-CHE). However, it is important to point out that Labs-BIO (1–4) were the smallest (Table 1) and had lower size/occupancy ratios, so CO₂ built up more. The lowest values for CO₂ concentration were measured in Lab-ECO and Lab-CHE.4, which is in concordance with the largest size/occupancy ratio of these labs, 51 and 41 m³/person, respectively. Other works have also measured the CO₂ levels in different university laboratories (Valavanidis and Vatista 2006; Ugranli et al. 2015; Hussin et al. 2017; Telejko 2017; Kwong et al. 2019; Sahu and Gurjar 2020; Idris et al. 2020), finding that high occupancy and inadequate ventilation are the main reasons for an increase in CO₂ concentration during practical sessions.

Spanish regulation allows CO₂ concentrations in indoor air of 350 ppm for computer rooms and 500 ppm for laboratories above the measured outdoor air concentration (RD 2007), which was 441 ppm on average, in this study. As shown in Table 4, in Labs-CHE (1–3) and Labs-BIO (1–4), the CO₂ levels exceeded the regulatory limits, on average during 66% and 86% of the measurements, respectively. In addition, the CO₂ concentrations were higher than 1,000 ppm, the recommended threshold by ANSI/ASHRAE (2019) and between 34 and 63% of the measurements in these laboratories, although they were below the limit value of 5,000 ppm, which is considered a safe value limit for healthy adults in an eight-hour workday (www.osha.gov). Seppanen et al. (1999) reported that increased indoor CO₂ levels were positively associated with a statistically significant increase in one or more prevalent SBS symptoms. In this context, environmental comfort, which includes T, RH, and CO₂ concentration, was not totally satisfactory during some monitoring days. Consequently, the results of this study suggest the risk of SBS symptom appearance in the students, especially during hours with higher occupant density. Adequate natural ventilation or the use of mechanical ventilation devices would be advisable to reduce the obtained results (Ugranli et al. 2015).

### Table 4: Values established as the limit in the Spanish Normative for indoor environments (UNE-EN 16798-, (2020)) and percentages of measurements in each laboratory with values exceeding them

| T (°C) | R.H. (%) | CO₂ (ppm) | CO (ppm) | TVOCs (μg/m³) | O₃ (ppm) | PN₀.₅ (part/cm³) | PN₅ (part/cm³) | Bacteria (CFU/m³) | Fungi (CFU/m³) |
|-------|---------|-----------|----------|---------------|---------|----------------|---------------|----------------|----------------|
|      | Limit (%) | Limit (%) | Limit (%) | Limit (%) | Limit (%) | Limit (%) | Limit (%) | Limit (%) | Limit (%) |
| CHE.1 | 21–23 | 3 | 40–50 | 53 | 1–C₂<350 | 50 | 9 | 4 | 0 | 0 | 35.2 | 3 | 0.2 | 0 | 35.2 |
| CHE.2 | 27 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| CHE.3 | 35 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| CHE.4 | 42 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| BIO.1 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| BIO.2 | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| BIO.3 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| BIO.4 | 37 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| PC.1 | 23 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| PC.2 | 29 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ECO | 37 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

During the colder months, the heating systems dry outdoor air, so the RH value for indoor air drops below the recommended value. Low RH can cause SBS symptoms such as eye irritation, throat irritation, and coughing, and it increases the susceptibility to infectious diseases and asthma (Ratodi et al. 2017).
Chemical indoor air quality indicators

Indoor CO, O₃, TVOCs, and particle concentrations are considered chemical indicators of IAQ, and therefore, were analysed and the results from different labs were compared (Table 1).

The CO concentrations from all the laboratories were very low, with values below the limit of detection (LOD) for 62% of the monitoring days. For the remaining, the levels were between 0.25 and 11.50 ppm, with an average value of 1.95 ± 2.93 ppm. Figure 3c and Table 1 summarise the CO measurements for each laboratory, which were like those reported for other teaching laboratories (Valavanidis and Vatista 2006; Idris et al. 2020). No statistically significant differences were found among the laboratories since the performed activities did not contribute to increasing CO concentration except the occasional use of Bunsen burners in Lab-BIO.3. This pollutant is a combustion product, and its presence in laboratories, when burners are not used, indicates an infiltration issue from the outdoor environment (2.55 ppm of average CO concentration outdoors). In this way, the presence of CO could be associated with the burning of pruning and biomass residues, very common in the surrounding areas during the monitoring periods. The results of this study are consistent with those of other reported works in teaching laboratories where the CO levels increased because of combustion processes, such as traffic emissions, the use of Bunsen burners, and other combustion activities (Valavanidis and Vatista 2006; Hussin et al. 2017; Kwong et al. 2019; Idris et al. 2020).

Otherwise, only four laboratories in a single measurement reneged on the limit value of 9 ppm established in national legislation (UNE 2014) or 10 ppm set by the WHO (WHO 2010) (Table 4). Therefore, CO concentrations could be discarded as an SBS risk for the students.

Similar results were obtained for O₃ concentrations, with 68% of the measurements showing concentrations below the LOD. When O₃ was detected, the values did not exceed 0.032 ppm, with an average value of 0.008 ± 0.005 ppm (Table 1 and Fig. 3c). These results are like those obtained for undergraduate laboratories of Athens University (0.002 ppm of average concentration) (Valavanidis and Vatista 2006). Comparing the occupied and unoccupied laboratories’ mean values, it was observed that the occupancy and activity in the labs hardly affected the O₃ values. Thus, considering that O₃ is a photochemical pollutant, its presence indoors was probably due to its infiltration from the outside. With respect to the air quality limits, no national legislation refers to indoor ozone, but the recommended value of 0.2 ppm (UNE 2014) was never reached.

Only chemical and biological laboratories showed TVOC values above the photo-ionisation detector LOD during some monitoring days. Daily TVOC concentrations fluctuated because chemical reagents were used irregularly, depending on experimental schedules. The average concentrations for these labs were between 255 ± 411 μg/m³ and 1,002 ± 889 μg/m³ (Table 1 and Fig. 3d), in concordance with those reported for other laboratories (Kwong et al. 2019; Idris et al. 2020).

Lab-CHE.1 and Lab-BIO.2 had the highest TVOC levels, with peak concentrations of 3,625 and 3,525 μg/m³, respectively, values statistically different from those of the remaining labs (p-values < 0.05). Discrepancies among authors have been observed for TVOC levels, and while Valavanidis and Vatista (2006) or Idris et al. (2020) reported values similar to or even higher than those in our study (until 7,500 μg/m³), for the labs of the Department of Chemistry at the University of Athens (Greece) or Engineering Department at University Malaysia Terengganu, Rumchev et al. (2003) reported much lower values (29 μg/m³) for 15 university laboratories at Curtin University of Technology, Perth (Australia). The high TVOC levels measured in our laboratories reflected the impact on IAQ of chemicals used during the laboratory experiments. Concretely, Lab-CHE.1 is dedicated to experimental teaching in organic chemistry, where the use of chemicals such as acetone and hexane, among others, was frequent. Likewise, in Lab-BIO.2, the experimental teaching in cell cultures implied the use of ethanol as a disinfectant product. Although in both laboratories the fume ventilation hoods were switched on during sampling, this exhausting system seems to be insufficient to evacuate all indoor TVOCs.

To date, Spain has no specific legislation for indoor TVOC, but healthy guidelines used as a reference point out a comfort value for TVOC < 200 μg/m³ and a safe limit < 3,000 μg/m³ (UNE 2014). Therefore, it was found that, although the mean values did not exceed the safe limit, when TVOCs were detected, their concentrations were always above the comfort value. In addition, the safe limit value (3,000 μg/m³) was exceeded in 6% and 9% of the measurements (Table 4) in Lab-CHE.1 and Lab-BIO.2, respectively, involving an important health risk situation.

Fine-mode particles (in our study, those of a diameter less than 0.3 and 0.5 μm: PN₀.₃ and PN₀.₅) and those including a portion of coarse mode (diameter < 5 μm: PN₅) were measured in every laboratory, and their PN concentrations (particles/cm³) are displayed in Fig. 3e. Regarding PN₀.₃, the mean values for Labs-BIO (1–4) (20.41 ± 12.56 particles/cm³), Lab-ECO (22.92 ± 12.94 particles/cm³), and Labs-PC (1–2) (23.48 ± 13.03 particles/cm³) were slightly lower than that of Labs-CHE (1–4) (29.63 ± 19.54 particles/cm³). Among the labs of the last group, Lab-CHE.1 and Lab-CHE.3 stood out with the highest average values: 30.77 ± 15.42 and 36.04 ± 22.01 particles/cm³, respectively (Table 1).

PN₀.₅ concentrations were lower than those of PN₀.₃ (Fig. 3e), and just like before, the highest concentrations were measured in Labs-CHE (1–4) (average concentration
6.93 ± 9.71 particles/cm³), followed by Labs-BIO (1–4) (3.86 ± 4.06 particles/cm³), Labs-PC (1–2) (3.51 ± 2.09 particles/cm³), and Lab-ECO (3.41 ± 2.32 particles/cm³). However, no statistically significant differences were found for both fine-mode particles (PN₀.₁ and PN₀.₅) attending to the use of the laboratory (p-value > 0.05). This could be because the values in Lab-CHE.4 were lower than those for the remaining Labs-CHE, likely caused by a higher size/occupancy ratio and better particle dispersion, resulting in a lower mean value for Labs-CHE with respect to the others (Labs-BIO, Labs-PC, and Lab-ECO) (p-value < 0.05).

In addition, it is interesting to highlight that for both types of particles, similar values were obtained for monitoring carried out when the labs were occupied and unoccupied, with a ratio between both values close to one (Fig. 4). Therefore, the occupancy in the laboratories seems to have little effect on the observed variations.

A potential source of these fine-mode particles could be the secondary reactions favoured by the chemical environment of the laboratories—that is, the appearance of condensation processes from gas-phase compounds (gas molecules with low equilibrium vapour pressure condensing on a particle) (Steinfeld 1998). This process would be favoured by the presence of numerous chemical products stored inside the laboratories. Thus, in Labs-CHE, where the use of solvents was higher, the PN₀.₁ and PN₀.₅ levels were also higher.

The PN concentrations of coarse particles (PN₅) were lower than those of fine particles in all the analysed laboratories (Table 1). Higher values were reported for ultrafine particle concentrations (diameter < 0.1 μm) in undergraduate laboratories (average value = 21,694 particles/cm³) (Rumchev et al. 2003). A direct comparison is not possible because the particle sizes are very different. However, the particle number decreases when their diameter increases because of the coagulation of ultrafine and fine particles to give rise to coarse particles. This has been also observed for measured PN in the classrooms of France (Canha et al. 2016).

A post hoc Tukey ANOVA test indicated statistically significant differences for PN₅ (p-value < 0.05) depending on the use of the laboratory (Fig. 3f). Concentrations for Labs-CHE (1–4) and Labs-BIO (1–4)—with average values of 0.10 ± 0.09 and 0.09 ± 0.06 particles/cm³, respectively—were higher than those for Lab-ECO and Labs-PC (1–2), which had the same average value of 0.04 ± 0.03 particles/cm³ (Fig. 3f). In addition, there were important differences for PN₅ concentrations between monitoring from occupied and unoccupied labs, with a ratio value > 12 (Fig. 4). This pattern is associated with the indoor generation of coarse particles by the occupants themselves, and has been observed in similar studies (Canha et al. 2016; Steinfeld 1998; Ugranli et al. 2015).

Fine and coarse particles differ not only in size and morphology but also in formation mechanisms, dosimetry (deposition in the respiratory tract), toxicity, and chemical, physical, and biological properties (Wilson et al. 2002), so their variations and sources may also differ. Moreover, it is important to indicate that fine particles travel more from outdoors compared to coarse particles and suspend for longer periods in the environment (Mishra et al. 2020). A priori, here, a possible source for coarse particles could be from the presence of people in the laboratories since they would produce skin and hair fragments or the resuspension of settled dust on the floor by their movement (Ugranli et al. 2015). Therefore, the increment of PN₅ concentrations could be due to the suspended dust in the room, and additional movements of the occupants exaggerate the conditions. Moreover, the levels of these particles in Labs-CHE were the highest, so not only were there primary sources, but also, the coarse particles could come from secondary oxidative processes favoured by the presence of chemical compounds (Steinfeld 1998; UNE 2014).

Finally, it should be noted that PN₀.₅ and PN₅ concentrations sporadically exceeded the recommended (not compulsory) values in the Spanish Quality Standards for Indoor Environments (PN₀.₅: 35.20 particles/cm³; PN₅: 0.29 particles/cm³) (UNE 2014) (Table 4). Until now, no national standard value exists for PN₀.₁ concentration.

**Fig. 4** Ratio of mean values for indoor particles and airborne microorganisms, obtained from occupied and unoccupied laboratories, according to their use.
Human health risk assessment

HQ values were used to estimate non-carcinogenic risks from exposure to some indoor chemical pollutants (see Table 3). The VOC risk assessment was calculated for specific solvents with known RFC (https://www.epa.gov/risk/regional-screening-levels-rsls-generic-tables), such as hexane or acetone (see Table 3). In these cases, only the days in which these solvents were used in the laboratories were considered for the calculation of HQ. Similarly, the frequency of exposure (FE) for CO and O₃ was lower than the usual teaching period (50 days per course), since these pollutants had values below the LOD for 62% and 68% of the samples, respectively.

HQ values for each of the pollutants evaluated were less than 0.1, even for organic compounds with a very high average concentration, and the accumulated HQ (or HI) was lower than the limit of 0.1 (0.04). Therefore, it can be concluded that there is not a significant non-carcinogenic risk due to inhalation of chemical contaminants in the laboratories studied. However, it is important to mention the carcinogenic risk posed by acute short-term exposure to certain chemical contaminants, such as VOCs (Guo et al. 2004), for which Li et al. (2021) reported to have neurological and carcinogenicity effects by inhalation.

Indoor airborne microorganisms

Microorganisms in indoor air are from activities by occupants, contaminated building materials, furnishings, and outdoor air. Monitoring of the concentration and composition of bioaerosols is increasingly being considered in IAQ analysis because of their influence on the health of the inhabitants. In general, indoor bacterial concentration is affected by the type and number of people located in a specific area, while indoor fungal concentration depends on the indoor moisture conditions, cleaning frequency, and outdoor infiltration and, therefore, atmospheric conditions.

In this study, both bacteria and fungi counts were carried out in all the labs using two sampling techniques: an impaction sampler and the gravity method. Figure 3g and h shows the mean values for counts of both types of microorganisms from both sampling methods. In general, counts from the impaction method were higher. So, the bacterial counts ranged between 221 CFU/m³ (Lab-BIO.2) and 860 CFU/m³ (Lab-CHE.2) from the impaction method and between 24 CFU/plate/h (Lab-PC.1) and 103 CFU/plate/h (Lab-CHE.1) from the gravity method. The fungi values were between 71 CFU/m³ (Lab-BIO.4) and 467 CFU/m³ (Lab-ECO) from the impaction method and between 6 CFU/plate/h (Labs-CHE.2, BIO.2 and PC.1) and 62 CFU/plate/h (Lab-ECO) from the gravity method. These results agree with those of other authors (Park et al. 2014; Pasquarella et al. 2000) who reported that when both sampling methods were used (impaction versus gravity), counts from active air monitoring were higher than those from passive ones.

It is important to point out that the comparison of counts from both methods would not be fully consistent because the gravity method does not allow the measuring of air volume sampled and is considered a non-quantitative method. Nevertheless, this method is still widely used because it is inexpensive and simple to use, and in the opinion of different authors (Di Giulio et al. 2010; Pasquarella et al. 2000), the results obtained are reproducible and reliable, constituting an effective system to monitor the microbial quality of environments. Nowadays, it is accepted that both methods can be used for the general monitoring of air contamination, such as routine surveillance programs.

For all the labs, the ranges of counts and mean values for bacteria were higher than those for fungi, independent of the sampling method, except for Lab-ECO, wherein the values for fungi counts from the impaction method were slightly higher. Other authors (Di Giulio et al. 2010; Jo and Seo 2005; Pastuszka et al. 2000) report similar results, finding that fungal concentration constituted less than 20% of the total count of microorganisms in the indoor air of the buildings.

It is well-known that the comparability of indoor air microbial counts from different studies is not an easy task because the results vary from study to study given many simultaneously influencing factors such as room size, occupancy, activity, sampling, and cultivation methods. Di Giulio et al. (2010), in a study analysing airborne microflora in university laboratories, reported values for fungi always lower than 50 CFU/h/plate, in concordance with our results. On the other hand, Jurado et al. (2014) reported values for viable fungi between 367 and 1,001 CFU/m³ when analysing classrooms in five Brazilian universities, higher than those in our study. On the contrary, counts reported for the indoor air of different facilities (library, lecture halls, laboratories, etc.) in a Nigerian university were lower, ranging between 4 and 440 CFU/m³ for bacteria and between 1 and 57 CFU/m³ for fungi (Amengual et al. 2017). Finally, those reported by Stryjakowska-Sekulska et al. (2007) for the air in chemical laboratories in a Polish university ranged between 110 and 650 CFU/m³ and 90 and 520 CFU/m³ for bacteria and fungi, respectively, in concordance with the results obtained in this study.

As reported by some authors (Mandal and Brandl 2011), in non-industrial indoor environments, one of the most important sources of airborne bacteria is the presence of human beings. Some activities such as talking or walking—both of special relevance in a university environment, where very often, a high number of students cohabit in a space of reduced dimensions—could generate an increase of airborne biological PM. When values for counts and those of the size/occupancy ratio were compared (Table 1), an
inverse relationship was observed. For instance, Labs-BIO (1–4) had the lowest values for this ratio, and three of them (Lab-BIO.1, Lab-BIO.3, and Lab-BIO.4) had the highest bacterial counts, while Lab-ECO, with the highest ratio, had one of the lowest bacterial counts.

Other factors significantly affecting the levels of indoor bioaerosols are the RH and T (Pasanen et al. 2000), but the values for these parameters measured in the labs were very similar (Table 1), and the observed differences were not statistically significant, as stated above. Therefore, these indoor environment comfort indicators would not be responsible for the differences in the microbial counts observed among the labs.

In addition, related to the growth of microorganisms in indoor air is the total particle concentration, and Agranovski et al. (2004) affirm that it might be used to trace viable bioaerosol particles. To this respect, Labs-CHE (1–4) and Labs-BIO (1, 3–4)—having high PN$_{0.3}$, PN$_{0.5}$, and PN$_{5}$ concentrations—also had high bacterial counts. Luoma and Batterman (2001) affirmed that fungal counts correlate only with particles between 1 and 5 µm, while Hargreaves et al. (2003) obtained a correlation with the total number of particles <2.5 µm. These results disagree with those obtained in our study because the highest values for fungal counts corresponded to Lab-PC.2 and Lab-ECO, and in them, the particle number concentrations were the lowest.

Considering the use of the labs, it was observed that Labs-BIO (1, 3–4) had the highest bacterial counts, which could be related with the material used during practical lectures, such as Petri dishes containing microbial cultures and soil and water samples. Moreover, Lab-ECO had the highest fungal count, which could also be related to the plant materials or other sources used for experimentation activities.

The comparison of counts obtained in the occupied and unoccupied labs displayed values for the ratio higher than one for all the labs and for both types of microorganisms. Higher ratio values were obtained for bacteria counts using the gravity method (Fig. 4). It is well accepted that most of the bacterial species present in indoor air are from human presence (Bonetta et al. 2010; Mentese et al. 2009; Tsai and Macher 2005), while fungal growth depends mainly on moisture and the availability of carbon sources. This would explain why bacterial counts were higher in occupied labs and why the ratio values were higher for bacteria than for fungi counts.

Spain has no specific legislation for indoor microbial contamination, and only recommended values for bacterial and fungal counts, using the impaction method, have been reported (UNE 2014). These values are <600 CFU/m$^3$ and <200 CFU/m$^3$ for bacteria and fungi counts, respectively. The percentages of measurements wherein these values were exceeded at each lab are shown in Table 4. Labs-CHE (1–4) and Labs-BIO (1–4) exceeded the regulatory limits for bacterial counts by an average of 47% and 43% of the sampled days, respectively, while the values for Lab-ECO and Labs-PC (1–2) exceeded the regulatory limits at a much lower percentage of days. For fungal counts, the average percentages of days exceeding limits ranged between 8% for Labs-CHE (1–4) and 21% for Labs-PC (1–2).

Indoor/outdoor ratio

Indoor air pollutants may originate from indoor sources or infiltrate from the outdoor environment. Therefore, calculating the ratio between the indoor and outdoor levels (I/O) of all the studied parameters makes it possible to determine the different sources of pollutants affecting IAQ (Table 5). As expected, the indoor CO$_2$ levels were higher than the outdoor levels, with an I/O ratio higher than 1.5, suggesting that the occupants’ respiration and metabolism are the key source of indoor CO$_2$ (Yau et al. 2012). On the contrary, both the CO and O$_3$ I/O ratios were less than one. As mentioned

| Activity                      | Chemistry and Chemical Engineering | Biology   | Computer | Ecology |
|-------------------------------|-----------------------------------|-----------|----------|---------|
|                               | CHE.1    | CHE.2    | CHE.3    | CHE.4    | BIO.1    | BIO.2    | BIO.3    | BIO.4    | PC.1    | PC.2    | ECO |
| I/O CO$_2$                    | 2.1*     | 2.0      | 1.9      | 1.5      | 2.2      | 2.1      | 2.3      | 2.3      | 1.8     | 1.8     | 1.5 |
| CO                            | 1.0      | 0.8      | 0.7      | 0.8      | 1.0      | 0.3      | 1.0      | 0.4      | 0.2     | 0.7     | 0.6 |
| O$_3$                         | 0.2      | 0.2      | 0.3      | 0.4      | 0.1      | 0.2      | 0.2      | 0.1      | 0.4     | 0.4     | 0.3 |
| PN$_{0.3}$                    | 1.4      | 1.1      | 1.5      | 1.0      | 0.9      | 0.7      | 1.0      | 0.8      | 0.8     | 1.2     | 1.0 |
| PN$_{0.5}$                    | 2.1      | 1.5      | 2.4      | 1.2      | 0.9      | 0.6      | 1.0      | 1.3      | 0.7     | 1.1     | 0.8 |
| PN$_{5}$                      | 1.8      | 2.1      | 1.7      | 0.9      | 0.6      | 1.8      | 1.5      | 1.5      | 0.6     | 0.6     | 0.6 |
| Bacteria impaction            | 1.8      | 2.2      | 1.5      | 1.7      | 1.9      | 0.6      | 1.8      | 1.9      | 0.7     | 1.1     | 0.8 |
| Fungi impaction               | 0.2      | 0.2      | 0.2      | 0.2      | 0.3      | 0.2      | 0.4      | 0.2      | 0.2     | 0.6     | 0.7 |
| Bacteria gravity              | 0.5      | 0.4      | 0.4      | 0.4      | 0.4      | 0.5      | 0.4      | 0.4      | 0.2     | 0.3     | 0.3 |
| Fungi gravity                 | 0.2      | 0.1      | 0.2      | 0.2      | 0.2      | 0.1      | 0.2      | 0.1      | 0.1     | 0.2     | 0.5 |

*Values highlighted in red represent I/O ratios above 1
above, indoor activity in the labs did not give rise to these pollutants, but come from external sources, such as biomass burning for CO and outdoor photochemical reactions for O₃. The I/O ratio for TVOC levels could not be calculated in this study because the outdoor measurements were always less than the LOD. However, as stated above, the presence of VOCs in the laboratories was due to indoor sources, such as the use of solvents in certain experimentation activities. Regarding particles, the I/O ratios varied from 0.6 to 2.4 for fine particles (PN₀.₃ and PN₀.₅) and from 0.6 to 2.1 for coarse particles (PN₅). The occupancy and people’s activities have been reported in several studies as the dominant factors for higher I/O ratios for coarse particles (Elbayoumi et al. 2013; Goyal and Kumar 2013). In this sense, I/O ratios for PN₅ were higher than one in laboratories with low size/occupancy ratios, such as Labs-BIO (2–4), which could indicate that indoor sources related to the people’s presence (skin and hair fragments or the resuspension of settled dust by movement) were accentuated in this type of space. For fine particles, the highest I/O values were in chemistry laboratories. A potential source for them would be the condensation from gas-phase compounds and gas molecules with low equilibrium vapour pressure condensing on a particle (Pöschl 2006). This process would be favoured by the presence of VOCs given the solvents used in these spaces, as mentioned above.

The I/O ratio for both the bacteria and fungi counts from the impaction method were assessed, and different trends were observed. The fungi ratios were lower than one in all the labs, while those for bacteria were higher than one for 8 of the 11 laboratories analysed, indicating an indoor source for bacteria. This confirms that the presence of bacteria in indoor air was mostly attributable to people since humans contain a high quantity of bacteria on/in their bodies that can be expelled to the air during usual activity, while airborne indoor fungi come from outdoor sources. Moreover, Jurado et al. (2014) affirm that I/O ratios above one can be the consequence of inappropriate cleaning practices and insufficient ventilation.

Stryjakowska-Sekulska et al. (2007) established three grades for the I/O ratio to determine IAQ: I/O ≤ 1.5 = good; I/O = 1.5–2.0 = regular; and I/O > 2 = poor indoor ambient conditions. Using this scale and considering the I/O ratio for bacteria, the indoor air of 5 out of the 11 sampled labs would have good quality, and for the remaining would be considered regular. However, if the I/O ratios for fungi were considered, all the labs would be qualified as good.

**Ventilation rates**

As indicated in the methods section, ventilation effectiveness can be calculated from the decay in CO₂ concentration. Figure 5 shows the ACH and VR values for the studied laboratories. The ACH value varied between 0.4 and 0.5 h⁻¹ and therefore, only half of the indoor air was removed for one hour. A previous study (Klein et al. 2009) indicated that ACH values below 6 h⁻¹ should only be considered for laboratories using small quantities of non- or low-hazard organic solvents since low ventilation requires exceptionally long times for indoor air to be cleared. Therefore, the situation was particularly worrying at Labs-CHE and Labs-BIO, due to high TVOC concentration measurements during several monitoring days.

Consequently, the VRs were also low (averaged value of 3.0 ± 0.5 L/s × person), although these values varied depending on the laboratory size and the number of students. In
this sense, Lab-ECO and Lab-CHE.4, with a high size/occupancy ratio (Table 1), had VR values above 5 L/s × person, higher than those obtained in the remaining labs (≤ 3.1 L/s × person). In general, the VRs obtained in this study were lower than those obtained in other university laboratories, where mechanical ventilation was used (10–65 L/s × person) (Sahu and Gurjar 2020), so the use of a small grille on the laboratory wall as a natural ventilation system was clearly insufficient. This poor ventilation system, with doors and windows closed, promoted low VRs in the laboratories when the occupancy was higher.

Global standards and guidelines related to minimum laboratory VRs are inconsistent. For instance, in the United States, ANSI/ASHRAE (2019) recommends a minimum VR of 5 L/s × person for computer and science laboratories. In Europe, European Standard UNE-EN (2020) specifies a minimum VR of 20 and 14 L/s × person in spaces with air quality I (laboratories) and II (computer rooms), respectively. In Spain and based on this European normative, the minimum recommended VR ranges are between 12.5 and 20.0 L/s × person for computer rooms and laboratories, respectively (RD 2007). According to our results, all the studied spaces presented VR mean values below the recommended thresholds from the European Standard UNE-EN (2020) and Spanish guidelines (UNE 2014), and only two laboratories were above the less restrictive value of 5 L/s × person, recommended by ANSI/ASHRAE (2019). Therefore, the use of the grilles situated on the walls and the fume ventilation hoods as the only laboratory ventilation systems is clearly insufficient, and solutions should be sought to achieve effective air renewal during practical teaching.

A temporary but easy solution would be the adoption of recommendations for windows and doors to be opened during breaks to increase air change in the laboratory. However, in adverse weather conditions, the widespread use of manual airing does not usually guarantee a decreased outdoor air pollution, nor does it ensure the conditions of hygrothermal comfort and energy consumption (Stabile et al. 2019; Alonso et al. 2021). The problem of poor environmental conditions is especially serious in indoor spaces in countries with a Mediterranean climate, such as Spain, with manual opening of windows as the only ventilation system to try to achieve good IAQ (Alonso et al. 2021). Indeed, it is expected to worsen in the climate change scenarios forecast (CEN 2008). Increasing outside air fractions in a Mediterranean climate entails a rise of total air change rates, but it may also lead to higher energy consumption (Alonso et al. 2021). In particular, when airing periods leading to the air exchange rate required by standards are adopted, the CO₂ concentration can decrease to values lower than 1000 ppm, but the ventilation losses increase up to 36% of the overall energy need for space heating of the room (Stabile et al. 2019). On the contrary, when the same air exchange rate is applied through mechanical ventilation systems equipped with heat recovery units, the ventilation energy loss contribution decreases to 5% and the overall energy saving results higher than 30%. For this reason, undoubtedly, the best solution would be to install adequate mechanical ventilation with a heat recovery unit and air filter system to control the air flow that enters and leaves the laboratory, expelling contaminated air to the outside and ensuring a distribution of clean air inside, which provides environmental comfort and energy saving. This system would be especially recommendable for laboratories where chemical solvents are often used. Finally, the size/occupancy ratio of the labs should be considered to avoid overcrowding the smallest laboratories. Higher occupancy densities of the laboratory imply higher required air exchange rates that lead to longer airing periods for manual airing scenarios and unnecessary energy consumption.

Correlations

A correlation analysis of all the magnitudes discussed above was performed to determine the relationships among them and the results are shown in Fig. 6.

Occupancy correlates positively with CO₂ and the latter, in turn, with CO, TVOCs, PN₅, and both bacteria and fungi counts from the impactation method. This result displays the relationship between the increase of concentrations for some pollutants and the increase of occupancy and activity in the laboratory, in agreement with Madureira et al. (2016), making it necessary to implement adequate ventilation to provide a healthy and comfortable environment for the occupants.

On the contrary, O₃ correlates negatively with CO₂, TVOCs, and PN₅, supporting their outdoor and indoor origins. Moreover, O₃ is a very strong oxidising microbial agent used for the elimination of toxic and harmful microorganisms in the air (Moccia et al. 2020). This would explain the negative correlations between fungal impactation counts and O₃ concentration, even though this was always below 0.032 ppm in the air of the sampled labs. Surprisingly, the same effect was not observed for bacterial counts, which could be explained by the presence of airborne sporulated bacteria. It is well known that fungal spores are part of the normal life cycle of fungi, and therefore, are less resistant to chemicals and adverse environmental conditions than bacterial spores (Eissa et al. 2014).

Occupancy and the resuspension of previously deposited particles strongly influence the indoor concentrations of airborne particles (Blondeau et al. 2005). In this work, fine and coarse particles correlate with each other, which indicates, at least in part, a common origin. However, PN₀.₃ and PN₀.₅
do not correlate with occupancy and CO₂ concentration, and therefore, the origin of this type of particle may not be exclusively the activity and occupation of the laboratories. In this sense, fine particles can be formed from the secondary oxidative processes of the organic material in the laboratory, particularly in the presence of O₃ and NOₓ (Wilson et al. 2002). Moreover, there was a positive correlation between the bacterial and fungi counts from the impaction method and the particle concentrations of the three sizes measured, possibly reflecting poor ventilation in the laboratories.

T and RH are factors favouring microbial growth, and therefore, a correlation between both the indoor environment comfort indicators and the microbial counts was expected. The results in our study showed no correlation between T and bacteria and fungi counts, independent of the sampling method used, and the correlations with RH were weak (p-value < 0.05), in concordance with the findings of other authors (Chan et al. 2008; Lee et al. 2002; Madureira et al. 2016).

Finally, it is interesting to highlight the positive strong correlations obtained for both bacterial and fungal counts from the impaction and gravity methods.

**Conclusions**

Analysis of IAQ in training labs is a matter of special interest because these workplaces have some peculiarities compared to other university spaces and even with research laboratories. Firstly, it is possible to find specific pollutants in high concentrations, such as solvents in chemical and biological laboratories, and microorganisms in laboratories where ecology or microbiology are being taught. Secondly, they often are presented as a closed, crowded environment (usually with a higher occupancy than in research labs), where students and teachers spend a significant proportion of their working time. Therefore, it is a cause of health concern to determine their IAQ in order to apply, if necessary, the adequate corrective measures.

In this work, the analysis of physical, chemical, and microbiological pollutants in the indoor air of 11 teaching labs and the outdoor air in the surrounding area during the two study periods has allowed us to determine both the origin of the pollutants and their influence on IAQ.
of the labs studied. In addition, CO₂ concentration, and occupancy data, as well as size space, were used to assess ventilation effectiveness. HQ and HI values were calculated to estimate the non-carcinogenic risk by inhalation of each of the pollutants and the global risk, respectively. All of that has given rise to a multidisciplinary and complete study on the assessment of the IAQ in university training labs and the effectiveness of their ventilation systems.

Overall, the averaged values of T, CO₂, O₃, PN concentrations, and fungi counts were within the European acceptable limit range. On the contrary, those of RH, CO₂, TVOCs, and bacteria exceeded the limit range on many of the monitoring days, being especially worrying situations in the Labs-CHE and Labs-BIO. Moreover, as expected, both the specific use of each laboratory and their occupancy influenced the IAQ and has been displayed that, for these types of buildings, natural ventilation is not enough to get an acceptable IAQ. Although HQ and HI were lower than 0.1 for all considered cases and thus, hence no significant non-carcinogenic risk by exposure is expected, it is important to consider the carcinogenic risk posed by acute short-term exposure to certain chemical contaminants, such as VOCs.

Therefore, installing a mechanical ventilation with heat recovery unit, and an adequate air purification system together with the fume hood ventilation systems, is prescriptive to prevent air pollution and the appearance of adverse health effects in university students and lectures, in addition to unnecessary energy losses. This fact could be the first step towards creating green campus environments and could help to support energy retrofits that are not limited to reducing energy consumption, but also include aspects related to air quality and environmental comfort.

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Data availability  All the necessary data generated or analysed during this study are included in this published article. However, the datasets for statistical analysis are available from the corresponding author on reasonable request.

Declarations

Ethics approval  The authors declare no ethics approval, consent to participate, and consent for publication.

Competing interests  The authors declare no competing interests.

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