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Compliance of indoor air quality during sleep with legislation and guidelines – A case study of Lisbon dwellings

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**“Compliance of indoor air quality during sleep with legislation
and guidelines – a case study of Lisbon dwellings”**

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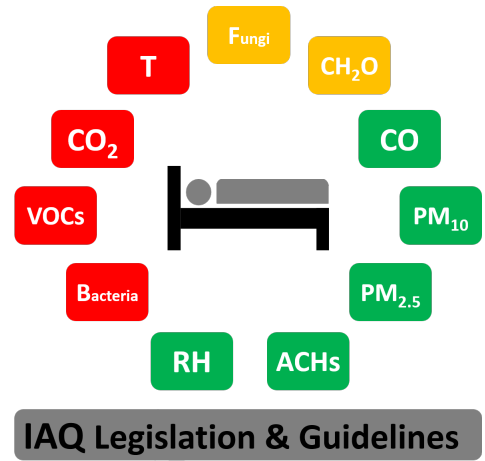
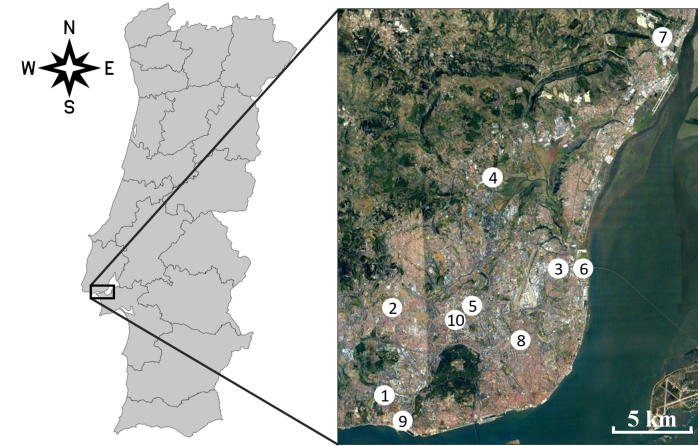
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1 **Compliance of indoor air quality during sleep with legislation and guidelines – a case study** 2 **of Lisbon dwellings**

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18 **Abstract**

19 This study aimed to provide a comprehensive characterisation of the indoor air quality during the sleeping period of 10 couples at Lisbon
20 dwellings, using a multi-pollutant approach, and to understand how the compliance with legislation and guidelines was to assure a good indoor
21 air quality. The assessment of indoor air quality was conducted in the cold season using real time monitors during the sleeping period for comfort
22 parameters (temperature and relative humidity) and air pollutants (carbon dioxide – CO₂, carbon monoxide – CO, formaldehyde – CH₂O, total
23 volatile organic compounds – VOCs, and particulate matter – PM_{2.5} and PM₁₀), together with active sampling of bioaerosols (fungi and bacteria)
24 before and after the sleeping period. Lower compliance (less than 50% of the cases) with the Portuguese legislation was found for temperature,
25 CO₂ (3440 ± 1610 mg.m⁻³), VOCs (1.79 ± 0.99 mg.m⁻³) and both bioaerosol types. In 70% of the cases, PM_{2.5} (15.3 ± 9.1 µg.m⁻³) exceeded the
26 WHO guideline of 10 µg.m⁻³. All bedrooms presented air change rates above the recommended minimum value of 0.7 h⁻¹, highlighting that a
27 good indoor air quality during sleep is not guaranteed.

28

29 **KEYWORDS**

30 Indoor air quality; sleep; exposure; particulate matter; bedroom; ventilation

31

32 **HIGHLIGHTS**

- 33 • PM_{2.5} levels during sleep exceeded the WHO guideline of 10 µg.m⁻³ in 70% of bedrooms
- 34 • Temperature, CO₂, VOCs and bacteria were above thresholds in 70% of bedrooms
- 35 • Ventilation guidelines are not enough to promote compliance with IAQ legislation

36

37 **1. Introduction**

38 Nowadays, indoor air quality (IAQ) is considered as a major factor that influences human health and the welfare of citizens (Sundell,
39 2004). This awareness is the result of years of research efforts, especially over the last two decades, focused on exposure levels in different
40 microenvironments and daily activity patterns. One of the main reasons for this shift in the exposure assessment studies, namely from outdoor to
41 indoor environments, was the awareness that, in developed countries, people spend around 90% of their time indoors (Almeida-Silva et al., 2014;
42 Faria et al., 2020).

43 Taking into account that people spend one third of their life sleeping (Canha et al., 2017) and that sleep is essential for human welfare,
44 performance and health (Krueger et al., 2016; Strøm-Tejsen et al., 2016), sleeping environments have started to gather some interest from the
45 scientific community in recent years (Boor et al., 2017; Katsoyiannis and Cincinelli, 2019; Lan and Lian, 2016) aiming at understanding the
46 exposure levels during sleep and how they can affect sleep quality.

47 The vital role of sleep in the human life is unquestionable. Multiple studies have shown its importance in many different spheres of the
48 daily life. For instance, a study conducted with university students in the United States of America (Becker et al., 2018) found that anxiety and
49 depressive symptoms were consistently associated with poorer sleep quality, with the former being linked with more sleep disturbances and sleep
50 medication use, and the latter with increased daytime dysfunction. Moreover, sleep difficulties led to lower academic results, increased daytime
51 sleepiness and emotion dysregulation.

52 Sleep duration also has been shown to have an impact on wellbeing, where short and long sleep duration was found to increase the risk of
53 health outcomes (Jike et al., 2018; Lubetkin and Jia, 2018), ranging from depression, poor cognition, and obesity, to cardiovascular disease,
54 including hypertension, coronary heart disease and stroke. A greater negative impact on morbidity and mortality was also associated with short
55 and long sleep duration (Jike et al., 2018; Lubetkin and Jia, 2018). The lowest mortality was experienced by participants reporting a usual sleep
56 duration of 7 hours (6.5-7.4 hours) per night (Kripke et al., 2002), which also corresponded to the lowest burden of disease in elderly people

57 (Lubetkin and Jia, 2018). However, the mortality hazard increased proportionately with sleeping duration, leading to an added mortality risk of
58 15% and an elevated hazard rate of cerebrovascular death of about 50% compared to people sleeping between 6 to 8 hours, both in men and
59 women (Alvarez and Ayas, 2004), when reported sleep exceeded 8.5 hours, or was below 3.5 hours for women and 4.5 hours for men (Kripke et
60 al., 2002). Sleep deprivation was also associated with the activation of the sympathetic nervous system, impairment of glucose control, increased
61 inflammation, higher cortisol levels, and reduced levels of leptin, an appetite-suppressing hormone, which may lead to weight gain and
62 eventually diabetes (Alvarez and Ayas, 2004). Moreover, sleep loss was also found to affect emotion regulation (Tempesta et al., 2018), lowering
63 emotional competence and empathy.

64 Several studies have already found that some environmental factors of the sleeping environments have a direct impact on the sleep quality
65 of the occupants, from comfort parameters (Lan et al., 2016; Pan et al., 2012; Zhang et al., 2018) and noise levels (Halperin, 2014) to ventilation
66 conditions, which are related to concentrations of pollutants, such as carbon dioxide (Strøm-Tejsen et al., 2016). However, sleeping environments
67 are still not fully characterised, considering the complexity of parameters that constitute indoor air, since most studies only focus on single
68 pollutants, such as CO₂ (Katsoyiannis and Cincinelli, 2019). The characterisation of IAQ in these specific micro-environments faces several
69 challenges, such as sleep disturbance due to noisy monitoring equipment (Canha et al., 2019). An IAQ overview during the sleeping period was
70 provided in a very few studies, in which it has been shown, for instance, that several pollutants (e.g. particulate matter, carbon dioxide,
71 formaldehyde and volatile organic compounds) can exceed the established guidelines (Almeida-Silva et al., 2014; Canha et al., 2017, 2019). A
72 wider knowledge about the air quality that people breathe during sleep is needed in order to understand which parameters can influence sleep
73 quality and how it can be improved by decreasing individuals' exposure.

74 The aim of the present study is to contribute to a comprehensive characterisation of indoor air quality of sleeping environments, based on
75 a multi-pollutant approach (comfort parameters, chemical and biological contaminants), which is essential for future calculation of exposure
76 levels and identifying the most critical parameters for the occupants. Therefore, this study characterised IAQ during sleep, in real conditions, of
77 bedrooms of ten couples from Lisbon (Portugal), using a real time monitoring strategy, assessing its compliance with legislation and guidelines.

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80 **2. Materials/Methods**

81 **2.1. Study site and individual's characterisation**

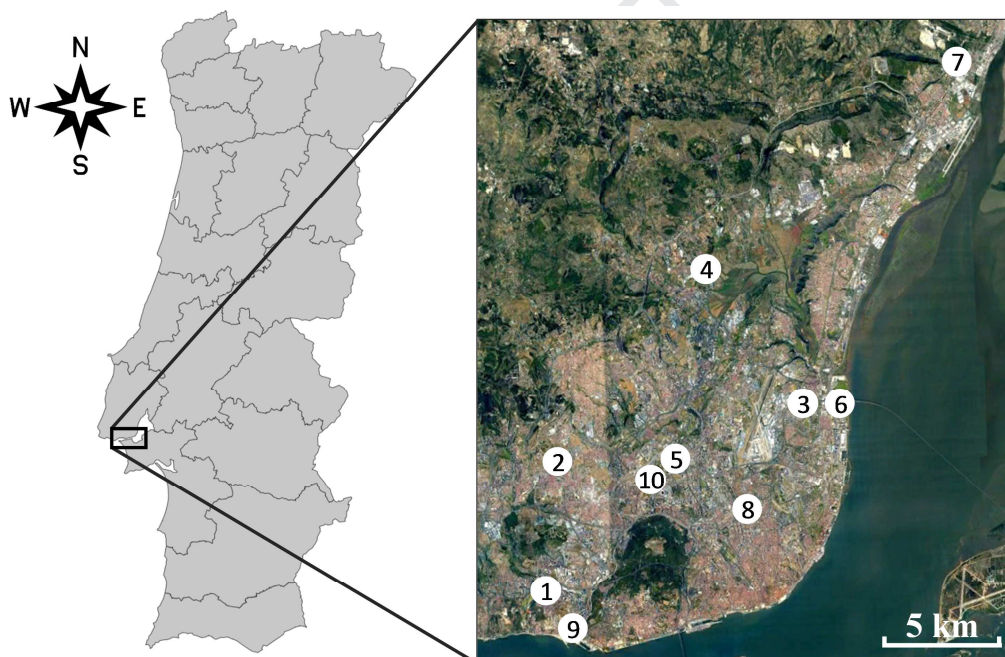
82 Ten volunteer couples participated in an IAQ monitoring programme during the 2016/2017 cold season in the urban area of Lisbon, Portugal.

83 Figure 1 presents the location of the dwellings where the study was conducted. All dwellings were apartments, located in different floors (varying

84 from second to eleventh floor). Table S 1 and

85 Table S 2 (both in “Supplementary Information” section) present details about the studied
86 dwellings and bedrooms, respectively.

87 The volunteers were selected to minimise confounders/external factors that could influence
88 the results. Therefore, the following selection criteria were applied: couples (male-female)
89 with ages between 25 and 45, without children with ages below 5 years, healthy (no
90 consumption of medication), no sleep disorders history and non-smokers. Table S 3 gives
91 details about the volunteers that participated in this study. The volunteers were requested to
92 sleep in usual conditions, namely regarding the ventilation patterns. All of them always slept
93 with the bedroom window closed, but some kept the door open while others closed it (only
94 couples 5 and 8 usually slept with the bedroom door closed).



95
96 Figure 1. Location of the studied dwellings in the Lisbon district, Portugal.

97 **2.2. Indoor air quality monitoring**

98 The monitoring programme was based on a comprehensive multi-pollutant assessment where
99 physical (temperature and relative humidity), chemical (carbon dioxide, carbon monoxide,
100 formaldehyde, volatile organic compounds, particulate matter – PM_{10} and $PM_{2.5}$) and
101 microbiological (fungi and bacteria) parameters were quantified. The IAQ monitoring was

102 conducted during three nights relying on typical real-time instruments (Canha et al., 2017,
103 2019):

104 1) Carbon monoxide (CO₂), temperature (T), relative humidity (RH) and total volatile
105 organic compounds (VOCs), by using a Graywolf (IQ-610 probe, WolfSense
106 Solutions, USA);

107 2) Formaldehyde (CH₂O), by using a Formaldemeter (htV-M, PPM Technology, UK);

108 3) Particulate matter of aerodynamic diameter lower than 2.5 µm (PM_{2.5}) and 10 µm
109 (PM₁₀), by using a DustTrak DRX monitor (8533 model, TSI, USA).

110 All the devices were calibrated according to the manufacturers' specifications and the
111 sampling frequency was set to 60 seconds. Due to the noise emitted by the pump of the
112 DustTrak DRX monitor and in order to avoid interference with the sleep quality of the
113 volunteers, a soundproofed wooden box was created to place this equipment in it. Figure S 1
114 ("Supplementary Information" section) shows the apparatus of IAQ monitoring in the
115 bedrooms. The monitoring devices were placed at the centre of the bedroom, at approximately
116 one meter from the bed and at about 80 cm from the floor, since this height corresponds
117 reasonably to the breathing level of a person lying in bed.

118 Despite the factory calibration of the DustTraks used in our study, the response of optical
119 instruments may vary for different types of aerosols (Moosmüller et al., 2001), which makes
120 the calibration process of the utmost importance for accurate measurements. Therefore, PM_{2.5}
121 and PM₁₀ readings of the used DustTrak instruments were rectified using correction factors
122 obtained from an inter-comparison study using these instruments and reference gravimetric
123 equipment from Leckel (Berlin, Germany). The Leckel samplers are certified by the European
124 Committee for Standardisation as a reference instrument for PM₁₀ and PM_{2.5} measurements
125 according to CEN EN 12341 and CEN EN 14907. This inter-comparison study was done in an
126 office with low occupancy. Figure S 2 provides the inter-comparison results for both PM
127 fractions, using the two different measurement methods. Reasonable to good correlations were
128 obtained between the DustTrak and Leckel data (with R² ranging from 0.56 to 0.78). PM_{2.5}
129 and PM₁₀ concentrations measured by DustTrak were corrected based on the linear regression
130 equations shown in Figure S 2. One of the real time instruments over-reports the PM₁₀ and
131 PM_{2.5} mass concentrations by a factor up to 1.7. This value is in line with previous studies
132 comparing gravimetric or filter-based methods with DustTrak measurements. A study in an

133 indoor environment impacted by biofuel combustion reported a factor of 1.65 for PM_{2.5}
134 measurements with DustTraks (McNamara et al., 2011), while in a wood smoke ambient
135 airshed, DustTraks over-recorded PM₁₀ by a factor of 2.73 (Kingham et al., 2006). Likewise,
136 DustTraks measured concentrations that were 1.94 – 2.57 times higher than filter-based and
137 federal reference method measurements in occupied and test homes (Ramachandran et al.,
138 2000; Wallace et al., 2011; Yanosky et al., 2002).

139 Microbiological counts were also assessed in each bedroom, before and after the sleeping
140 period (within a time frame of 30 minutes after the volunteers woke up). The sampling
141 procedure was based on active sampling using a MAS-100TM air sampler device. Colony
142 forming units (CFU.m⁻³) of bacteria and fungi were counted after an incubation period of 7
143 days at the typical temperature for each microorganism type. The methodology was already
144 fully described elsewhere (Canha et al., 2015).

145 The IAQ monitoring took place from December 2016 to March 2017 in each bedroom during
146 3 consecutive weeknights (from Tuesday to Thursday), during the usual sleeping period of the
147 volunteers. The monitoring period in each bedroom varied according to the usual sleeping
148 patterns of the volunteers, with a mean sleep night of 450 minutes per bedroom and the
149 sleeping period ranging from 22:00 to 09:20.

150 The mean results of the chemical and microbiological parameters for each bedroom were
151 evaluated taking into account the Portuguese legislation (*Ordinance no. 353-A/2013*) that
152 establishes 8-h limit values specifically for indoor air.

153

154 **2.2.1. Calculation of air changes per hour**

155 In order to characterise the ventilation of the bedrooms during the sleeping period, air changes
156 per hour (ACHs, h⁻¹) were calculated using the tracer gas method, i.e. the CO₂ emitted by the
157 occupants during the sleeping period and focusing on its build-up phase, through the
158 application of a computerised tool already described elsewhere (Hänninen, 2013). Examples
159 of its application can be found in the literature for different micro-environments, such as
160 classrooms (Canha et al., 2016; Hänninen et al., 2017), gyms (Ramos et al., 2014) and
161 bedrooms (Almeida-Silva et al., 2014; Canha et al., 2017).

162

163 2.3. Statistical analysis

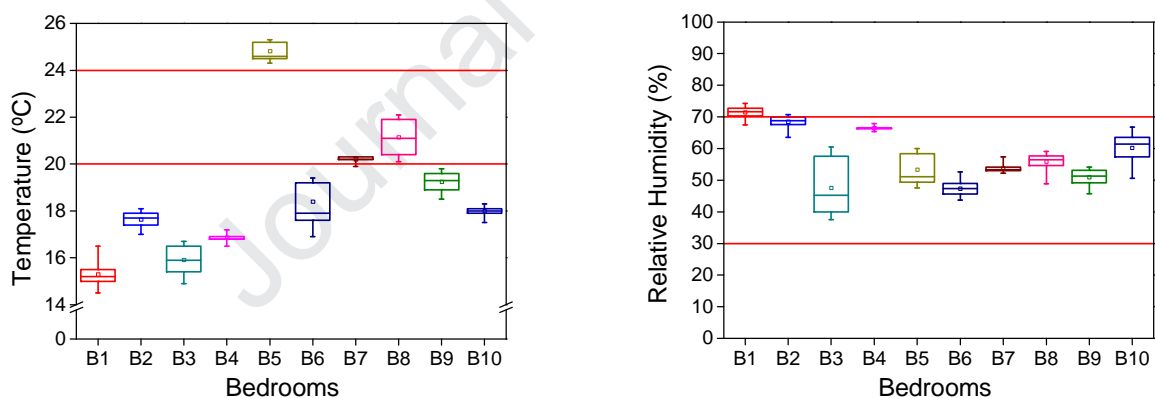
164 The statistical analyses were conducted using Excel and XLSTAT 2014.1.09 software
165 programmes. Non-parametric statistics were applied to the environmental monitored data,
166 namely, Spearman correlations to analyse potential associations between parameters. Origin
167 version 7.5 (OriginLab Corporation) was used to plot the results.

168 3. Results and Discussion

169 3.1. Comfort Parameters

170 3.1.1. Temperature

171 Figure 2 presents the temperature values registered during the sleeping period in the 10
 172 bedrooms, with a mean value of 18.8 ± 2.8 °C, ranging from 15.3 ± 0.4 (bedroom 1) to $24.8 \pm$
 173 0.3 (bedroom 5). Considering the international guideline ISO 7730:2005 (ISO, 2005) that
 174 establishes a temperature range for the occupants' comfort for the colder period between 20
 175 and 24°C, only two bedrooms provided mean temperatures within that range (bedrooms 7 and
 176 8). Most of the bedrooms (7 out of 10) presented mean values below the recommended range
 177 for thermal comfort, ranging from 15.3 ± 0.4 (bedroom 1) to 19.2 ± 0.4 (bedroom 9). Only
 178 bedroom 5 registered a mean temperature during sleep above the recommended guideline
 179 (24.8 ± 0.3 °C). Overall, only 20% of the cases were within the recommended range of
 180 temperature, which is below the 58% of cases found in a previous study conducted in 12
 181 bedrooms with single occupancy (Canha et al., 2019) in Portugal (as shown by **Error!**
 182 **Reference source not found.** in the Supplementary Information section).



183
 184 Figure 2. Temperature (left) and relative humidity (right) during the sleeping period in the 10
 185 bedrooms. Red lines represent the recommended range of values and the box plots show the
 186 25, 50 and 75 percentiles, with minimum, average (square) and maximum values.

187
 188 Most of the bedrooms presented colder temperatures than the comfortable range for sleep,
 189 which highlights the lack of heating during the colder period and a typical type of
 190 construction in Southern European countries that is not capable to deal with the extreme
 191 temperatures that can happen in winter and in summer. These results are in agreement with a
 192 study conducted in bedrooms of 141 dwellings in the North of Portugal, where a mean

193 temperature of 14.9 ± 3.1 °C was found, with only 4% of the dwellings with temperatures
194 during sleep above 20°C (Magalhães et al., 2016).

195

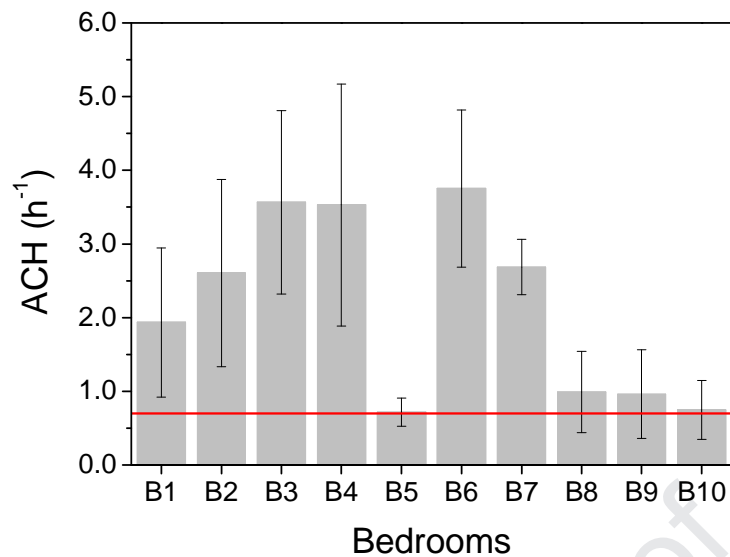
196 **3.1.2. Relative Humidity**

197 An overall mean of $57.6 \pm 8.7\%$ was registered for the 10 bedrooms (Figure 2). Taking into
198 consideration the recommended range for the occupants' comfort in indoor environments
199 (ISO, 2005), which was set at 30-70% in colder periods, only 1 bedroom presented a mean
200 value above the upper limit (bedroom 1, with a mean value of $71.4 \pm 1.6\%$), while all other
201 bedrooms have met the requirements. Similar results were found in the previous study
202 mentioned above that assessed IAQ in 12 bedrooms with single occupancy, in which relative
203 humidity mean values were within the recommended range. The high levels of relative
204 humidity in bedroom 1 during sleep may be due to the outdoor conditions, since there was
205 heavy rain during the monitoring period of that specific bedroom.

206

207 **3.2. Air Changes per Hour**

208 Figure 3 depicts the mean values of ACHs in each bedroom during the sleeping period and
209 Table S 4 ("Supplementary Information" section) provides details about their calculation and
210 a statistical summary. All bedrooms presented mean ACHs higher than the minimum value of
211 0.7 h^{-1} established for bedrooms by EN 16798-1:2019 (CEN, 2019). The global average was
212 $2.15 \pm 1.24 \text{ h}^{-1}$, ranging from $0.72 \pm 0.19 \text{ h}^{-1}$ (bedroom 5) to $3.75 \pm 1.06 \text{ h}^{-1}$ (bedroom 6).
213 Bedroom 6 was the only one with mechanical ventilation, which explains the higher value of
214 ACHs. Despite having natural ventilation, bedrooms 3 and 4 also had ACHs above 3 h^{-1} . The
215 range of ACHs registered in the present study is similar to those obtained in previous works
216 conducted in single occupancy bedrooms (Canha et al., 2017, 2019), which concluded that
217 opening the bedroom door is the main factor influencing ACHs during sleep.



218

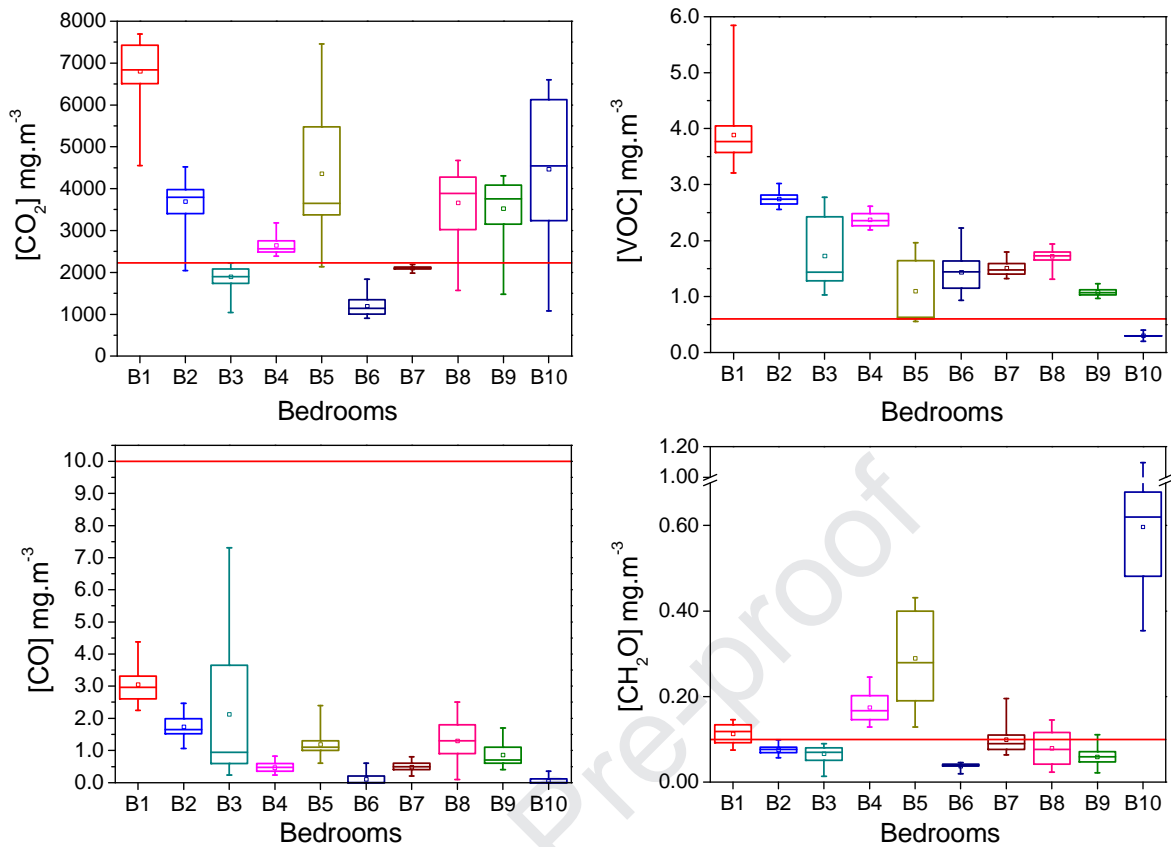
219 Figure 3. Air change rates for each bedroom during the sleeping period. Red line stands for
 220 the minimum value of 0.7 h^{-1} established for bedrooms by EN 16798-1:2019 (CEN, 2019).

221

222 3.3. Carbon Dioxide

223 Only 30% of bedrooms were below the limit value of 2250 mg.m^{-3} defined by the Portuguese
 224 legislation (*Ordinance no. 353-A/2013*) set to CO_2 concentration (Figure 4). Overall, the mean
 225 CO_2 level during sleep registered in this study was $3440 \pm 1610 \text{ mg.m}^{-3}$, ranging from $1200 \pm$
 226 210 mg.m^{-3} (bedroom 6) to $6810 \pm 660 \text{ mg.m}^{-3}$ (bedroom 1). The bedroom with the lowest
 227 CO_2 levels was the same that had the highest ACHs, i.e. the one with mechanical ventilation,
 228 showing the importance of this system in diluting air contaminants.

229



230
 231 Figure 4. Levels of CO₂ (top, left), CO (bottom, left), VOC (top, right) and CH₂O (bottom,
 232 right) during the sleeping period in each bedroom. Red lines represent the limit value
 233 established by the Portuguese Ordinance no. 353-A/2013 for each parameter. Box plots
 234 present 25, 50 and 75 percentiles, with minimum, average (square) and maximum values.

235 Carbon dioxide levels clearly indicates that the air change rates are not enough to promote its
 236 dilution (concurrently with other pollutants) during sleep, which can lead to a lower quality of
 237 sleep. As already described in the literature, high levels of carbon dioxide during sleep
 238 promote a lower sleep quality and next day performance (Strøm-Tejsten et al., 2016). A CO₂
 239 level of around 1500 mg.m⁻³ (835 ppm) was considered the threshold above which several
 240 parameters would be negatively affected, such as, sleep quality and next-day performance
 241 (Strøm-Tejsten et al., 2016), in a study with university's students in Denmark. In the present
 242 study, only one bedroom (bedroom 6) presented a mean CO₂ level during the sleeping period
 243 lower than this threshold. It is important to highlight that all bedrooms were occupied by two
 244 adults, which may explain the higher CO₂ levels found in the present study, when comparing
 245 to single occupancy studies, such as the ones previously conducted in Portugal. In single-unit
 246 dwellings, mean CO₂ levels were always below the limit value of 2225 mg.m⁻³ (Canha et al.,
 247 2017), while other study registered 67% of the cases (8 out of 12 bedrooms with single

248 occupancy) with mean values above the limit value and a peak mean CO₂ level of 4808 ±
249 1139 mg.m⁻³ (Canha et al., 2019). A study in Portuguese elderly care centres found that 40%
250 of the cases (4 in 10 bedrooms with single occupancy) had mean levels of CO₂ above the limit
251 value, peaking at 3000 mg.m⁻³ (Almeida-Silva et al., 2014). During the sleeping period, the
252 occupants are the only source of CO₂ and its generation rate per person depends of several
253 factors, such as the age, gender and other physiological parameters like the mean body mass
254 (Persily and de Jonge, 2017). On average, the CO₂ generation rate by a couple (one male and
255 one female) during sleep is 0.0036 L.s⁻¹.person (Persily and de Jonge, 2017).

256 A study conducted in Poland during the sleeping period of one female teenager (Mainka and
257 Zajusz-Zubek, 2019) showed that CO₂ levels of 6000 mg.m⁻³ were reached several times,
258 when the bedroom's door was closed. Another study carried out in Chinese student
259 dormitories reported a mean CO₂ steady value of 3150 mg.m⁻³ during sleep and associated
260 levels above 3384 mg.m⁻³ with worse IAQ satisfaction (Zhang et al., 2018).

261

262 **3.4. Carbon Monoxide**

263 The mean CO level was 1.14 ± 0.96 mg.m⁻³, ranging from 0.05 ± 0.07 mg.m⁻³ (bedroom 10)
264 to 3.05 ± 0.52 mg.m⁻³ (bedroom 1) (Figure 4). All bedrooms presented mean CO levels always
265 below the limit value of 10 mg.m⁻³ established by the Portuguese legislation (*Ordinance no.*
266 *353-A/2013*). Bedroom 1 was the one where CO levels were higher during sleep. This is likely
267 related to the location of the kitchen, which was next to the bedroom, and, therefore, some CO
268 infiltration may have occurred due to the emissions of the appliances that existed in there.

269 Carbon monoxide is typically a product of incomplete combustion processes that, in indoor
270 environments, can be originated by cooking appliances, water heating systems or fireplaces
271 (Canha et al., 2018; Mullen et al., 2016), or by infiltration from outdoor sources, such as
272 traffic exhaust emissions (Ramos et al., 2016).

273

274 **3.5. Formaldehyde**

275 Overall, the mean CH₂O level was 0.16 ± 0.17 mg.m⁻³, ranging from 0.04 ± 0.17 mg.m⁻³
276 (bedroom 6) to 0.60 ± 0.14 mg.m⁻³ (bedroom 10). Four bedrooms showed mean CH₂O levels
277 during the sleeping period above the limit value of 0.1 mg.m⁻³ established by the Portuguese
278 legislation (Figure 4). High levels of CH₂O indoors may be originated from emissions from
279 household materials and consumer products (Canha et al., 2017).

280 The results of the present study showed a high variability between the studied bedrooms but
281 are, in some way, in agreement with other results documented in the literature. A previous
282 study conducted in bedrooms of smokers and non-smokers in Portugal showed a significant
283 difference between the CH₂O levels in each type of bedroom during the sleeping period
284 (Canha et al., 2019): $0.11 \pm 0.03 \text{ mg.m}^{-3}$ (smokers) and $0.05 \pm 0.14 \text{ mg.m}^{-3}$ (non-smokers).
285 Another study that evaluated different ventilation conditions in a single room reported CH₂O
286 levels ranging from $0.090 \pm 0.034 \text{ mg.m}^{-3}$ (bedroom with closed door and open window) to
287 $0.205 \pm 0.082 \text{ mg.m}^{-3}$ (both door and window opened) (Canha et al., 2017). A study in 10
288 bedrooms of elderly care centres in Lisbon (Portugal) revealed mean CH₂O levels ranging
289 from 0.03 to 0.15 mg.m⁻³ (Almeida-Silva et al., 2014). In the USA a mean value of 0.017
290 mg.m⁻³ was found in 340 bedrooms (Mullen et al., 2016), in Spain a mean value of 0.027
291 mg.m⁻³ was monitored in 10 bedrooms (Rovira et al., 2016), in Gonabad (Iran) a mean level
292 of 0.149 mg.m⁻³ was registered in 20 bedrooms of new houses (Dehghani et al., 2018) and in
293 Shanghai (China) a mean level of 0.029 mg.m⁻³ was found in 20 bedrooms of asthmatic
294 children (Fang et al., 2019). Overall, the values of the studies conducted in Portugal are
295 slightly higher than the ones reported for other countries. In addition to possible variations in
296 sources and physical parameters that affect emissions (e.g. temperature), the use of different
297 measurement techniques can affect the comparability of results. In the present study, a real
298 monitoring device (Formaldemeter htV-M) was used. Its lower specificity for lower
299 concentrations ($<0.120 \text{ mg.m}^{-3}$) and potential interferences on the electrochemical sensor from
300 other VOCs (Hirst et al., 2011) may contribute to some inaccuracy of the results. Despite the
301 potential overestimation of CH₂O levels, this type of methodology has the advantage of
302 allowing the temporal variability of the pollutant during the sleeping period. Instead, standard
303 methods based on liquid impingers, coated-solid cartridges, or sorbent tubes, only provide a
304 value over the exposure time.

305

306 **3.6. Volatile Organic Compounds (VOCs)**

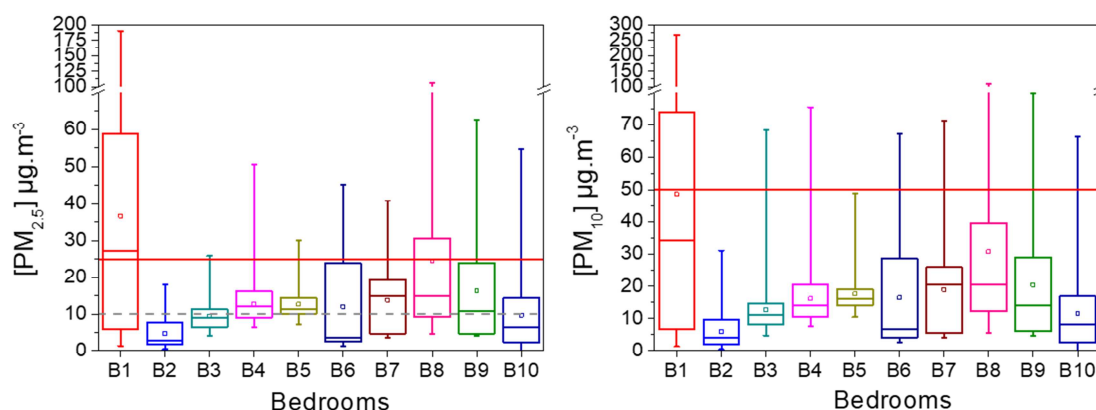
307 Only one bedroom (B10) presented a mean VOC level ($0.33 \pm 0.05 \text{ mg.m}^{-3}$) below the limit
308 value of 0.1 mg.m^{-3} established by the Portuguese Ordinance no. 353-A/2013 (Figure 4),
309 namely. The overall mean was $1.79 \pm 0.99 \text{ mg.m}^{-3}$, which is around 18 times higher than the
310 threshold, with the highest level being registered in bedroom 1 ($3.89 \pm 0.50 \text{ mg.m}^{-3}$). Similar
311 VOC patterns have already been found in previous studies, although of a lower magnitude,
312 with concentrations exceeding 5 times the recommended value (Canha et al., 2019). This

313 group of pollutants is emitted by common household products and building materials, such as
 314 paints and varnishes, as well as by cleaning and consumer products (Chin et al., 2014).

315 3.7. Particulate Matter (PM)

316 The overall PM_{2.5} mean ($15.3 \pm 9.1 \mu\text{g}\cdot\text{m}^{-3}$) was below the limit value of $25 \mu\text{g}\cdot\text{m}^{-3}$ established
 317 by the Portuguese legislation (Figure 5). PM_{2.5} concentrations ranged from $4.7 \pm 3.7 \mu\text{g}\cdot\text{m}^{-3}$
 318 (bedroom 2) to $36.6 \pm 36.8 \mu\text{g}\cdot\text{m}^{-3}$ (bedroom 1). Only bedroom 1 presented levels above the
 319 threshold during the sleeping period, although the mean value in bedroom 8 (24.4 ± 23.0
 320 $\mu\text{g}\cdot\text{m}^{-3}$) was also close to the legal limit.

321 The PM₁₀ mean levels registered in all bedrooms were also below the threshold of $50 \mu\text{g}\cdot\text{m}^{-3}$
 322 imposed by the Portuguese legislation, with an overall mean concentration of 19.9 ± 12.0
 323 $\mu\text{g}\cdot\text{m}^{-3}$, ranging from $5.9 \pm 5.1 \mu\text{g}\cdot\text{m}^{-3}$ (bedroom 2) and $48.6 \pm 50.4 \mu\text{g}\cdot\text{m}^{-3}$ (bedroom 1). A
 324 high proportion of PM₁₀ was composed of fine particles. PM_{2.5} accounted, on average, for 77
 325 $\pm 4\%$ of PM₁₀ levels (ranging from 72% in bedroom 5 to 84% in bedroom 10).



326
 327 Figure 5. PM levels during the sleeping period in the 10 studied bedrooms: (left) PM_{2.5} and
 328 (right) PM₁₀. Red lines represent the limit values established by the Portuguese Ordinance no.
 329 353-A/2013, whilst the dash grey line is the guideline value recommended by the World
 330 Health Organisation. Box plots present the 25, 50 and 75 percentiles, with minimum, average
 331 (square) and maximum values.

332 Coarse particles are typically associated with resuspension of mineral dust (Calvo et al., 2013)
 333 but, in indoor environments, a variety of human activities, such as cleaning or ironing, and
 334 other sources, such as textiles or human skin desquamation, contribute to the PM₁₀ levels
 335 (Alves, 2017; Morawska et al., 2017). Regarding the fine particles, 70% of the bedrooms
 336 showed mean levels above the guideline of $10 \mu\text{g}\cdot\text{m}^{-3}$ recommended by the World Health
 337 Organisation (EEA, 2018). It is important to highlight that WHO states that there is no
 338 evidence of a safe level of PM exposure or a concentration value below which no adverse

339 effects occur (WHO Regional Office for Europe, 2013) and, due to this awareness and
 340 scientific outcomes already achieved, in 2013, PM_{2.5} was classified as carcinogenic to human
 341 beings by the International Agency for Research on Cancer (IARC, 2013; Loomis et al.,
 342 2013).

343 Moreover, even if, according to the national legislation, the mean levels of PM can be
 344 considered relatively low, their impacts on occupants' personal exposure can be significant,
 345 given the time people spend in the bedroom (typically around 8h). A study conducted in
 346 Lisbon (Portugal) to assess children's exposure to particulate matter found that, after the
 347 classroom (PM_{2.5} - 42.4%; PM₁₀ - 49.7%), bedrooms are the micro-environment that
 348 contribute most to exposure (PM_{2.5} - 26.7%; PM₁₀ - 21.5%) (Faria et al., 2020), despite being
 349 the one with the lowest concentrations (always below 20 µg.m⁻³). The estimated exposure
 350 during the sleeping period in weekdays was 141 µg.m⁻³.h and 177 µg.m⁻³.h for PM_{2.5} and
 351 PM₁₀, respectively. Higher exposures were registered on weekends: 192 µg.m⁻³.h for PM_{2.5}
 352 and 245 µg.m⁻³.h for PM₁₀. As stated above, the high exposure to PM is due to the significant
 353 time spent in this micro-environment, which shows its importance to the overall human
 354 exposure.

355 The exposure (E) is defined by $E = C_j \cdot t_j$, where C_j is the PM concentration measured in a
 356 specific micro-environment and t_j is the time spent in it (Faria et al., 2020; Morawska et al.,
 357 2013). The potential inhaled dose (D) can be estimated by multiplying the exposure in a
 358 specific micro-environment by the inhalation rate (IR, m³.h⁻¹) of the occupants during that
 359 period. IR depends on the type of activity developed by the occupants and their age
 360 (Buonanno et al., 2011). For the volunteers of the present study, the IR for sleeping and
 361 resting can be assumed as 0.36 m³.h⁻¹ (age group between 19 and 40 years old) (Buonanno et
 362 al., 2011). Table 1 presents PM exposure and potential inhaled dose assessed for the
 363 bedrooms of the present study.

364 Table 1. PM_{2.5} and PM₁₀ exposure and correspondent potential inhaled dose during the
 365 sleeping period for the studied bedrooms.

Bedroom	Exposure (µg.m ⁻³ .h)		Potential inhaled dose (µg)	
	PM _{2.5}	PM ₁₀	PM _{2.5}	PM ₁₀
1	272.8	362.1	98.2	130.3
2	41.4	43.7	14.9	15.7
3	67.2	93.9	24.2	33.8

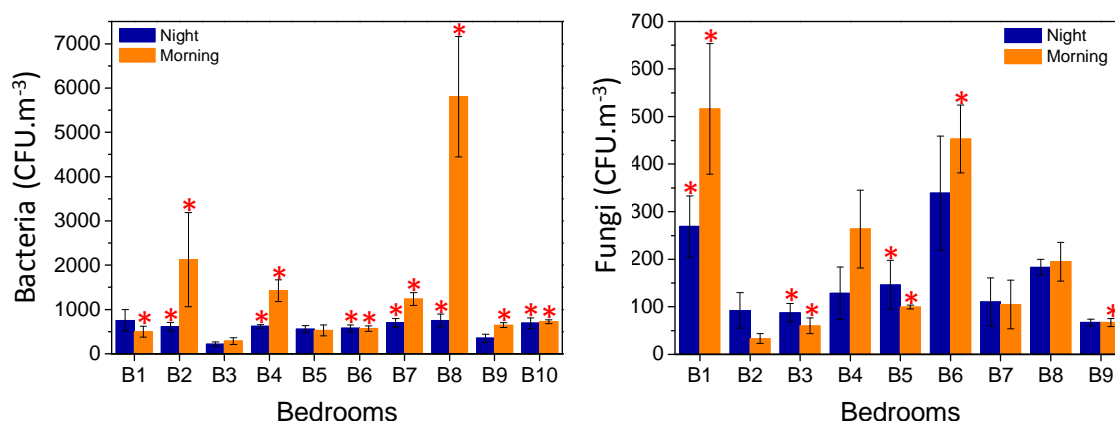
4	86.3	120.0	31.1	43.2
5	85.3	131.2	30.7	47.2
6	95.8	122.4	34.5	44.1
7	100.4	140.5	36.1	50.6
8	177.9	229.2	64.1	82.5
9	142.6	152.0	51.3	54.7
10	66.5	86.0	23.9	30.9
Mean \pm SD	113.6 \pm 64.8	148.1 \pm 84.8	40.9 \pm 23.3	53.3 \pm 15.7
[Min-Max]	[41.4 - 272.8]	[43.7 - 362.1]	[14.9 - 98.2]	[15.7 - 130.3]

366

367 Exposures to PM_{2.5} and PM₁₀ were estimated to be 113.6 \pm 64.8 $\mu\text{g}\cdot\text{m}^{-3}\cdot\text{h}$ and 148.1 \pm 84.8
368 $\mu\text{g}\cdot\text{m}^{-3}\cdot\text{h}$, respectively. These values are lower than the ones previously assessed for children
369 in Lisbon (Faria et al., 2020): 141.4 $\mu\text{g}\cdot\text{m}^{-3}\cdot\text{h}$ for PM_{2.5} and 177.4 $\mu\text{g}\cdot\text{m}^{-3}\cdot\text{h}$ for PM₁₀. The
370 mean potential inhaled doses during the sleeping period in the present study was 40.9 \pm 23.3
371 μg for PM_{2.5} and 53.3 \pm 15.7 μg for PM₁₀, which were close to the assessed potential inhaled
372 doses by children (43.8 μg for PM_{2.5} and 55.0 μg for PM₁₀) (Faria et al., 2020).

373 3.8. Bioaerosols

374 Levels of bioaerosols (bacteria and fungi) were quantified before (night) and after the sleeping
375 (morning) period in the 10 bedrooms (Figure 6). For fungi, due to operational and logistical
376 constraints, it was not possible to assess the loads in bedroom 10). The Portuguese legislation
377 establishes that the indoor bacteria levels should be lower than the sum of the outdoor level
378 and 350 CFU.m⁻³, while for fungi the indoor levels should be simply lower than the outdoor
379 levels (*Ordinance no. 353-A/2013*). For bacteria, in 80% of the cases, the morning levels were
380 above the limit value established by the national legislation. This is likely related to the fact
381 that humans are a source of bacteria (Canha et al., 2015). Regarding fungi, only 44% of the
382 bedrooms presented morning values below the corresponding outdoor loads. A morning/night
383 ratio of 1.12 \pm 0.57 was registered for fungi, while the corresponding value for bacteria was
384 2.21 \pm 2.10, highlighting the role of human occupancy as a driver of bacterial contamination
385 in the indoor air. The present study only quantified the colony forming units. However,
386 further work should be conducted in order to perform the identification of the different species
387 of fungi and bacteria in sleeping environments.



388
 389 Figure 6. Bioaerosols levels before (night) and after sleep (morning): (left) bacteria and (right)
 390 fungi. Red asterisks stand for cases above the limit values established by the Portuguese
 391 legislation (*Ordinance no. 353-A/2013*).

392 3.9. Spearman Correlations

393 Table S 5 (in the “Supplementary Information” section) shows the spearman correlations
 394 between the monitored parameters of indoor air during the sleeping period. The relative
 395 humidity was positively correlated with both CO₂ and bacterial loads. These two last
 396 parameters are both associated with the human presence, since CO₂ released through
 397 breathing (Strøm-Tejse et al., 2016), while several bacterial communities are shed by
 398 occupants (Hospodsky et al., 2012). A negative association between ACHs and CO₂ was
 399 found, as expected, since higher ACHs promote higher CO₂ dilution (Canha et al., 2016).
 400 Moreover, ACHs were calculated from CO₂ levels, which highlights this association.

401 A positive relationship between CO₂ and CH₂O, already described in other studies (Canha et
 402 al., 2016), was also observed, indicating that formaldehyde is emitted by indoor sources, such
 403 as building materials and consumer products (WHO, 2010).

404 A positive association between CO and VOCs was also found, highlighting their common
 405 source, such as infiltration of traffic emissions (von Schneidmesser et al., 2010). PM_{2.5} and
 406 PM₁₀ correlate well between each other, which is not surprising, as it was found that a
 407 significant part of PM₁₀ is composed of fine particles. As expected, the fungi levels before and
 408 after the sleeping period also presented a positive association, since no local source of fungi is
 409 present in the bedroom during the sleeping period. This is line with the morning/night ratio of
 410 1.12 ± 0.57 , previously mentioned.

411

412 3.10. Considerations

413 The use of real time monitors to assess temporal variability of pollutants during sleep is a
 414 good strategy to overcome some problems that can arise from reference methods, such as the
 415 gravimetric method for particulate matter, due to the noise of the sampling pumps and its
 416 potential interference in the sleep of the volunteers. However, despite all precautions on the
 417 use of such equipment (such as calibrations), results should be critically evaluated since
 418 potential under and over-estimation may be found, as already discussed and described
 419 elsewhere (Canha et al., 2019, 2017). Anyway, this multi-pollutant strategy allows a
 420 comprehensive assessment of the indoor air quality during sleep, with minimum impact on the
 421 volunteers' sleep, and provides meaningful insights about the compliance with guideline
 422 values, information that is scarce in the literature (Lan and Lian, 2016). The present work, as
 423 summarised in Table 2, shows that the indoor air quality during sleep in couples' bedrooms
 424 during winter time presents non-conformities for different parameters. On average, indoor air
 425 quality during sleep was within acceptable ranges only for 61% of the parameters,
 426 highlighting the need to focus on such type of environments and the need to apply preventive
 427 and remedial measures to breathe healthier air.

428
 429 Table 2. Compliance of IAQ for 11 parameters in the studied bedrooms with Portuguese
 430 legislation and other guidelines (*). "Yes" when the parameter is within the acceptable range
 431 or "No" if otherwise.

Bedroom	IAQ Parameters											% Yes
	T*	RH*	ACHs*	CO ₂	CO	CH ₂ O	VOCs	PM _{2.5}	PM ₁₀	Bacteria	Fungi	
B1	No	No	Yes	No	Yes	No	No	No	Yes	No	No	27%
B2	No	Yes	Yes	No	Yes	Yes	No	Yes	Yes	No	Yes	64%
B3	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	No	73%
B4	No	Yes	Yes	No	Yes	No	No	Yes	Yes	No	Yes	55%
B5	No	Yes	Yes	No	Yes	No	No	Yes	Yes	Yes	No	55%
B6	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No	No	64%
B7	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No	Yes	82%
B8	Yes	Yes	Yes	No	Yes	Yes	No	Yes	Yes	No	Yes	73%
B9	No	Yes	Yes	No	Yes	Yes	No	Yes	Yes	No	No	55%
B10	No	Yes	Yes	No	Yes	No	Yes	Yes	Yes	No	n/a	60%
% Yes	20%	90%	100%	30%	100%	60%	10%	90%	100%	20%	44%	

432

433 Some other limitations of the present study include the limited number of bedrooms (10) and
434 the reduced monitoring period (only 3 nights per bedroom). However, the information
435 gathered allows a first understanding of how IAQ can vary during the sleeping period of a
436 couple in a typical bedroom of a dwelling in Lisbon. To have a more robust characterisation
437 of this type of micro-environment, future efforts should be conducted to increase the number
438 of bedrooms. Moreover, further work should also be carry out to understand which
439 environmental factors may have impact on sleep quality, taking into account the importance
440 of nigh rest in the quality of human life.

441

442 **4. Conclusions**

443 The bedrooms under study presented an overall compliance of 61 ± 15 % with the guidelines,
444 ranging from 27% (bedroom 1) to 82% (bedroom 7). The parameters that fully met the
445 mandatory requirements in all bedrooms were only ACHs, CO and PM₁₀. The parameters that
446 showed a lower compliance (less than 50% of the cases) were temperature (30%), CO₂ (30%),
447 VOCs (10%), and bioaerosols, namely, bacteria (20%) and fungi (44%), together with PM_{2.5}
448 (30%), when considering the WHO guideline.

449 Air change rates, despite always being above the established guideline (0.7 h^{-1}), are clearly not
450 enough to provide a good indoor air quality during sleep by promoting the dilution of the
451 pollutants emitted. Therefore, a clear evidence from this work is that air change rates are not
452 enough to ensure compliance of pollutant levels with legal standards and guidelines. Lower
453 air quality may lead to lower sleep quality, as already described in the literature for CO₂ levels
454 and comfort parameters, which, in turn, will promote a degradation of the human welfare and
455 performance during daytime.

456

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643 **7. Supplementary Information**

644 Table S 1. Details of the studied dwellings located in the district of Lisbon, Portugal.

Dwelling	Location			Construction Year (recent renovation)	Area (m ²)	Height (m)	Typology, T _n *	Type of ownership	Type	Floor level	Type of house heating	Fuel to cook	Type of Ventilation	Type of flooring	Window	
	Parish	Municipality	Type of frame												Type of Glass	
1	Linda-a-Velha	Oeiras	1960 (2015)	52	2.8	T1	Rented	Apartment-type	2	Electric heaters	Gas	Natural ventilation	Wood	Aluminum	Double	
2	Mina de Água	Amadora	2007	115	2.1	T2	Own	Apartment-type	3	Electric heaters	Electricity	Natural ventilation	Wood	Aluminum	Double	
3	Portela	Lisbon	1990 (2015)	120	2.7	T2	Own	Apartment-type	2	Electric heaters	Gas	Natural ventilation	Wood	Aluminum	Double	
4	Loures	Loures	2011	110	2.5	T2	Own	Apartment-type	8	Air conditioning	Electricity	Natural ventilation	Wood	Aluminum	Double	
5	Carnide	Lisbon	2005	95	2.8	T2	Rented	Apartment-type	4	Oil heaters	Gas	Natural ventilation	Wood	Aluminum	Double	
6	Parque das Nações	Lisbon	2007	180	2.8	T4	Own	Apartment-type	11	Central heating	Gas	Mechanical ventilation	Wood	Aluminum	Double	
7	Sobralinho	Vila Franca de Xira	2009	80	2.8	T2	Rented	Apartment-type	3	Electric heaters	Gas	Natural ventilation	Tiles	Aluminum	Double	
8	Alvalade	Lisbon	1970 (2008)	100	2.87	T5	Rented	Apartment-type	2	n/a	Gas	Natural ventilation	Wood	Wood	Simple	
9	Algés	Lisbon	1960 (2012)	75	2.8	T2	Rented	Apartment-type	3	Electric heaters	Gas	Natural ventilation	Wood	Aluminum	Double	
10	Carnide	Lisbon	1990 (2013)	82	2.6	T1	Rented	Apartment-type	4	Electric heaters	Gas	Natural ventilation	Wood	Aluminum	Simple	

645 * T_n where n is the number of rooms

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650 Table S 2. Details of the studied bedrooms.

Dwelling	Bedroom	Area (m ²)	Floor type	Windows					Doors			Area of carpets or rugs (m ²)
				Nr	Area (m ²)	Type of frame	Glass type	Outdoor	Nr	Area (m ²)	Door facing	
1	B1	12.2	Wood	1	1.37	Aluminum	Double	Street	1	1.41	Hallway + bathroom + kitchen	2.78
2	B2	20.0	Wood	1	2.80	Aluminum	Double	Street	1	1.60	Hallway	n/a
3	B3	15.8	Wood	1	2.80	Aluminum	Double	Street	1	1.60	Hallway	n/a
4	B4	15.2	Wood	1	2.86	Aluminum	Double	Square in between buildings	2	1.50	Hallway + bathroom	1.32
5	B5	12.0	Wood	1	2.80	Aluminum	Double	Street	1	1.50	Hallway	n/a
6	B6	18.0	Wood	1	8.00	Aluminum	Double	Street	1	1.60	Hallway	4.50
7	B7	10.5	Wood	1	2.62	Aluminum	Double	Street	1	1.50	Hallway	1.20
8	B8	20.0	Wood	2	4.72	Wood	Simple	Street	1	1.54	Hallway	n/a
9	B9	10.5	Wood	3	4.32	Aluminum	Double	Street	1	1.60	Hallway	n/a
10	B10	10.5	Wood	1	0.99	Aluminum	Simple	parking space and leisure park	1	1.40	Hallway	0.77

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655 Table S 3. Details of the participants in the study.

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Dwelling	Gender	Age	Height (m)	Weight (kg)
1	Female	28	1.63	50
	Male	29	1.86	83
2	Female	34	1.67	69
	Male	41	1.80	82
3	Female	29	1.61	60
	Male	31	1.74	70
4	Female	31	1.60	59
	Male	36	1.83	110
5	Female	26	1.65	66
	Male	26	1.81	74
6	Female	41	1.62	69
	Male	44	1.84	75
7	Female	26	1.64	64
	Male	26	1.73	73
8	Female	27	1.61	55
	Male	27	1.75	82
9	Female	30	1.66	51
	Male	31	1.80	70
10	Female	33	1.62	70
	Male	28	1.72	88

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658 Table S 4. Details of the calculation of air change rates and statistical summary for each studied bedroom.

Bedroom	Nr Nights	Nr CO ₂ events	Sleeping period (minutes)	Air Change Rates, h ⁻¹			
				Mean ± SD	Median	Min	Max
1	3	8	374	1.94 ± 1.01	1.64	0.96	3.77
2	3	15	568	2.60 ± 1.27	1.91	1.08	4.38
3	3	4	187	3.56 ± 1.25	3.71	2.04	4.80
4	2	5	209	3.53 ± 1.64	2.68	2.30	6.10
5	2	7	341	0.72 ± 0.19	0.65	0.46	0.96
6	2	8	277	3.75 ± 1.06	3.72	2.29	5.38
7	1	5	167	2.69 ± 0.38	2.59	2.34	3.28
8	3	12	618	0.99 ± 0.55	1.02	0.15	2.35
9	1	5	300	0.96 ± 0.60	1.11	0.32	1.62
10	2	11	433	0.75 ± 0.40	0.80	0.29	1.52

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673 Table S 5. Spearman correlations between indoor air parameters monitored during the sleeping period. Values in bold refer to significant
 674 correlations (p-value < 0.050).

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	T	RH	ACHs	CO ₂	CO	CH ₂ O	VOCs	PM _{2.5}	PM ₁₀	Fungi - N	Fungi - M	Bacteria - N	Bacteria - M
T	1	-0.39	-0.47	-0.05	-0.32	0.04	-0.62	0.19	0.27	0.04	-0.20	-0.05	0.32
RH		1	-0.25	0.71	0.26	0.56	0.56	0.20	0.01	0.15	0.22	0.70	0.39
ACHs			1	-0.78	-0.01	-0.55	0.47	-0.25	-0.22	0.02	0.14	-0.13	-0.08
CO ₂				1	0.26	0.66	0.05	0.22	0.15	0.22	0.13	0.45	0.03
CO					1	-0.18	0.67	0.16	0.28	-0.19	-0.32	0.03	-0.27
CH ₂ O						1	-0.08	0.09	-0.07	0.22	0.27	0.44	0.09
VOCs							1	0.09	0.05	0.04	0.08	0.33	0.07
PM _{2.5}								1	0.95	0.28	0.49	0.55	0.05
PM ₁₀									1	0.30	0.39	0.42	-0.12
Fungi - N										1	0.83	0.58	-0.03
Fungi - M											1	0.60	-0.09
Bacteria - N												1	0.42
Bacteria - M													1

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680 Figure S 1. Apparatus of IAQ monitoring during sleep with detail of the soundproofed wooden box and the real-time monitors.

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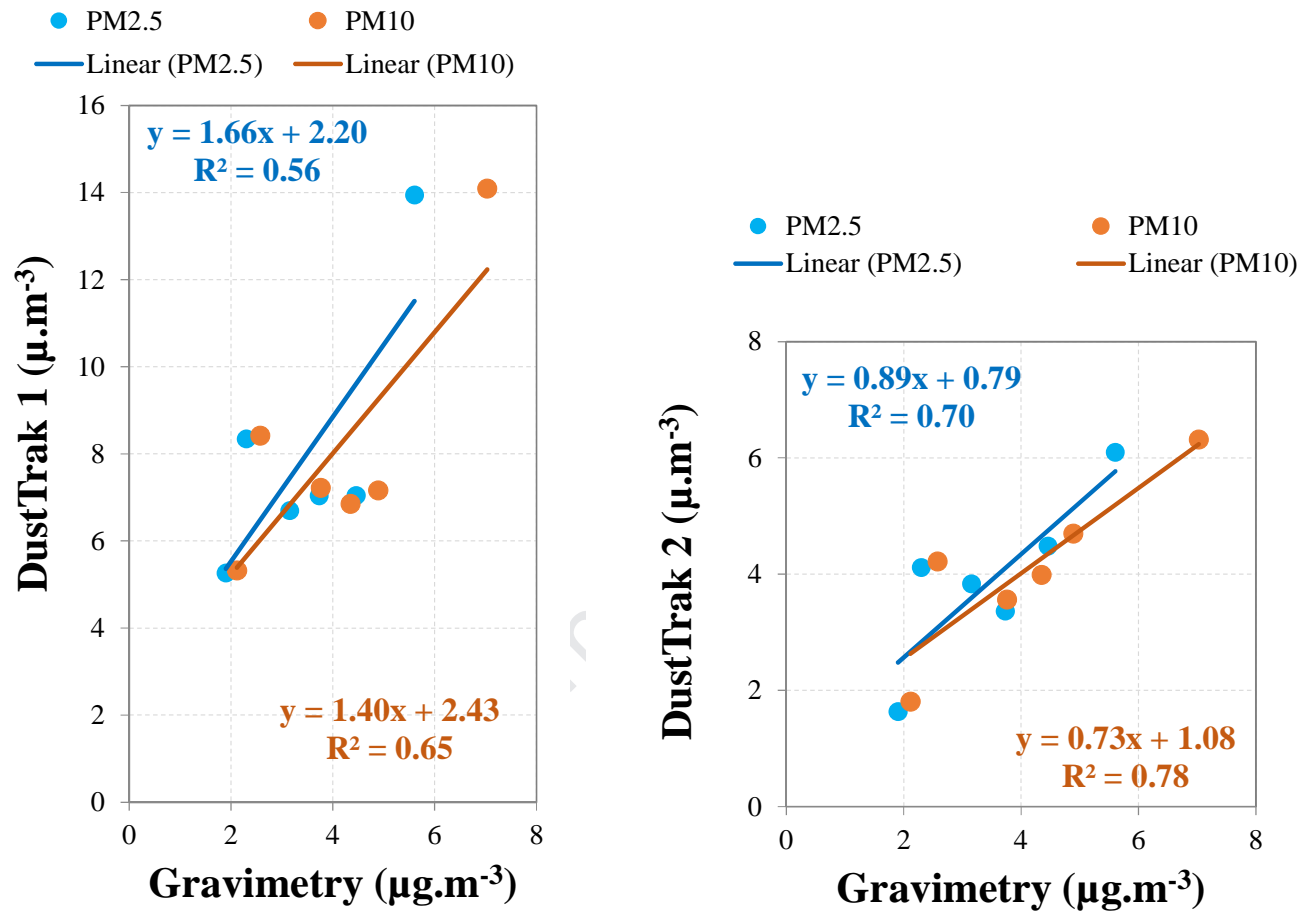
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Figure S 2. Inter-comparison exercise of measuring PM concentrations ($\mu\text{g}\cdot\text{m}^{-3}$) with the two DustTrak devices used in this study and the gravimetric method in an indoor environment.

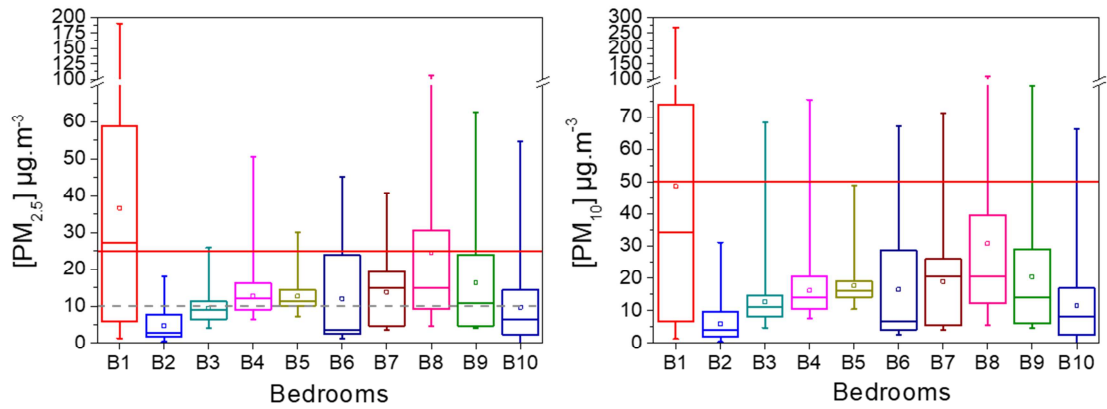


Figure 1. PM levels during the sleeping period in the 10 studied bedrooms: (left) PM_{2.5} and (right) PM₁₀. Red lines represent the limit values established by the Portuguese Ordinance no. 353-A/2013, whilst the dash grey line is the guideline value recommended by the World Health Organisation. Box plots present the 25, 50 and 75 percentiles, with minimum, average (square) and maximum values.

Compliance of indoor air quality during sleep with legislation and guidelines – a case study of Lisbon dwellings

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HIGHLIGHTS

- PM_{2.5} always exceeded the WHO guideline of 10 µg.m⁻³ during the sleeping period
- Temperature, CO₂, VOCs and bacteria were above thresholds in 70% of bedrooms
- Ventilation guidelines are not enough to promote compliance with IAQ legislation