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Culturable Bioaerosols Assessment in a Waste-Sorting Plant and UV-C Decontamination

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Abstract: Waste-Sorting Plant (WSP) workers are exposed to bioaerosols containing a large variety of bacterial and fungal species, posing a critical health risk that needs to be assessed and mitigated. The present study aimed to evaluate the indoor air quality in a Portuguese WSP and the air decontamination efficiency with UV-C. The concentrations of bacteria and fungi and particulate matter ($PM_{2.5}$ and PM_{10}), CO_2, relative humidity, and temperature were determined at different hours in manual sorting areas (cabin and ramp) in autumn and winter in 2022 and in administrative offices and canteen in the autumn of 2023. The $PM_{2.5}$ and PM_{10} concentrations in the air increased with the daily waste-sorting activities, especially inside the cabin, averaging 22 and 42 μg/m^3, respectively, while the CO_2 concentration was in the range of 343–578 ppm in both sampling sites. The bacterial species were mainly environmental (mesophilic bacteria) rather than human sources. In the waste-sorting areas, the concentration of bacteria was often found to exceed outdoor values by more than 1000 CFU/m^3 on average. Additionally, the concentration of fungi indoors was consistently higher than outdoor values, in many cases exceeding 500 CFU/m^3. These findings suggest that workers in these areas are frequently exposed to high levels of microbes. The indoor-to-outdoor (I/O) contamination ratios revealed that the air quality inside the administrative offices and the canteen had high pollutant concentrations during some time periods. The worst scenarios were observed in the canteen and offices with high occupancy in the afternoon. UV-C lamps at 253.7 nm and with 5.0 W irradiation power were used in the sorting cabin to test the indoor air and surface decontamination, and the results showed a high bacterial removal efficacy of over 87.6% after one hour of exposure to UV-C. The present study raises the question of whether 37 °C is the optimal incubation temperature for WSP samples since the microorganisms' habitat before the sampling had a much lower temperature. As the waste-sorting industry expands, these findings show that the air quality of WSPs remains concerning and requires a holistic approach, integrating the working conditions of all personnel and the implementation and monitoring of mitigation measures.

Keywords: waste-sorting plant; bioaerosols; air contamination; $PM_{2.5}$ and PM_{10}; carbon dioxide; indoor air quality; bacterial concentration; fungal concentration; UV-C decontamination

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1. Introduction

The United Nations' sustainability goals and the European Union Waste Framework Directive reflect the need for a transition towards a circular economy while addressing the increasing production of waste. As the waste-recycling industry expands and generates more jobs, it must be managed without harming human health or the environment [1]. A literature review, expert survey, and data collection on health problems and exposure to biological agents in the workplace was carried out by the European Agency for Safety and Health at Work between 2015 and 2017 and found that workers in waste treatment, waste collection, and composting are at risk of exposure to biological agents [2]. Additionally, it has been reported that activities such as waste management, the sorting of plastic, paper, glass, composting, and landfilling are sources of airborne bacteria, fungi, volatile organic compounds, and endotoxins [3–12]. Therefore, it is not surprising that the working conditions of the operators of the waste industry have received attention in many countries. Studies evaluating the fungal and/or bacterial air concentrations in a wastepaper- and cardboard-recycling factory in Tehran, a municipal waste-sorting plant in Poland, paper-sorting plants in Denmark, plastic waste-sorting facilities in the Czech Republic, a paper- and plastic-recycling plant in Portugal, and WSPs in France, Norway, Korea, and Brazil, among others, show through different analytical strategies that the operators are subjected to bioaerosols that may pose health risks [3,9,13–20]. The refuse disposal industry is listed in Directive 2000/54/EC [21] as one of the activities that could potentially expose workers to biological agents while on the job. These airborne biological agents, known as bioaerosols, may cause skin and eye infections, airway inflammation, respiratory disorders, allergic diseases, gastrointestinal problems, and symptoms of organic dust toxic syndrome [22–26]. Additionally, it has been proposed that waste-sorting activities may be a source of antibiotic resistance [27,28].

Particulate matter (PM) is released into the atmosphere due to waste-sorting activities, and the PM_{10} (diameter < 10 μm) and $PM_{2.5}$ fractions (<2.5 μm) are of particular concern as these particles are small enough to penetrate the thoracic region of the respiratory system and adversely affect respiratory and cardiovascular health [29–31]. These particles can carry microorganisms and transfer potential pathogens from the waste material to the airways of on-site workers. They can also be disseminated over larger areas, affecting nearby populations [25,30].

The establishment of occupational exposure limits has been hindered by the lack of data on the dose-response relationship and person-to-person variation in sensitivity and immunological reactions to harmful biological agents [32]. However, guidance values have been set in some countries; for example, in the Netherlands, there is a 90 EU/m^3 8 h time-weighted average (TWA) for endotoxin exposure and 1.5 mg/m^3 (8 h TWA) for inhalable grain dust [33] and a control value for spores of mesophilic molds of 5×10^4 spores/m^3 for waste management in Germany [34]. A synthesis of the guidance values proposed in different countries has been presented by Gorny et al., who indicate a range of $\leq 7.5 \times 10^2$–1.0×10^7 CFU/m^3 for the total number of bacteria, 1.0×10^3–2.0×10^4 CFU/m^3 for Gram-negative bacteria, and $<1.0 \times 10^2$–1.0×10^7 CFU/m^3 for fungi for productive and industrial purposes [32].

In Portugal, the institutions involving professional activities with an exposure risk to biological agents, like WSPs, wastewater treatment plants, the food industry, and others, must comply with Decree-Law no. 102-A/2020 as of 9 December [35]. This Decree-Law classifies the microorganisms according to their infectious risk degree and defines confinement measures and risk assessments, among other issues. The legislation concerning the energy systems and climatization of commercial and service buildings stipulates that the number of bacteria in the indoor air should not exceed the outdoor concentration plus 350 CFU/m^3, and the concentration of fungi should be lower than the outdoor concentration and not exceed 500 CFU/m^3 [36].

Bioaerosol viability in the air depends on factors such as relative humidity (RH), temperature, oxygen availability, ultraviolet radiation, and the type of organism [25]. For example, Gram-negative bacteria are viable for more extended periods at low-to-median RH levels, while Gram-positive bacteria remain viable at a high RH [25]. Several authors have reported seasonal and diurnal variations in bacterial and fungal air concentrations. For example, there were higher fungi concentrations in the summer and spring compared to autumn and winter and an increase in airborne fungi concentrations during the work shift in two plastic-sorting centers in the Czech Republic [9]. In a study of airborne bacteria in a wastepaper- and cardboard-sorting plant in Iran during the winter, bacterial concentrations were found to be positively correlated with the RH and negatively correlated with the temperature [19].

The methods available for bioaerosol control include filtration, ventilation, ultraviolet (UV) exposure, biocidal agents, physical isolation, and ion emissions, although not all may be applicable to waste-sorting activities [25,37,38]. Ultraviolet Germicidal Irradiation

(UVGI) is used to disinfect air, water, and surfaces and commonly employs a short-wave UV-C (100–280 nm), particularly at 254 nm [37]. Ultraviolet irradiation damages an organism's deoxyribonucleic acid (DNA), disrupting cell functions such as transcription, translation, and genome replication, leading to cell death [25,38]. The effectiveness of the control methods employed is influenced by several factors affecting survival, such as the type of organism and relative humidity; for example, the germicidal effectiveness of UV-C (253.7 nm) irradiation has been shown to be lower at 80% relative humidity compared to 50%, and an irradiance dosage difference of 80 times was observed for the inactivation of bacterial cells, *Escherichia coli*, and spores of *Penicillium citrinum* [39]. UVGI also has the advantage of low energy consumption and easy application. It can be used in combination with other methods, such as titanium oxide particles that cause photocatalysis [38]. A higher inactivation rate of *Escherichia coli* was observed at 185 nm when compared to 254 nm irradiance; the shorter wavelength was also reported to reduce the airborne endotoxic concentration, which the authors have attributed to the generation of ozone at a shorter wavelength treatment [40]. The application of UV light in dustbins for the attenuation of viruses and bacteria in the initial stages of waste handling has been proposed, and the use of UVGI for disinfection is employed in several contexts; for example, in hospital environments, the food industry, and wastewater treatment, and there is an increasing interest in its application in other settings due to the health crisis caused by the COVID-19 pandemic [37,41–46]. Other preventive measures proposed to control bioaerosols include the installation of clean and dirty locker rooms, negative pressure, ceiling air diffusers providing a downward airflow, and the use of respiratory protection equipment [47].

The WSPs in Portugal must comply with Decree-Law no. 102-A/2020 [35], which defines the minimum requirements for protecting the safety and health of workers against the risks of exposure to biological agents during work. As waste-sorting activities pose a risk of involuntary exposure to biological agents, the employer must carry out a risk assessment at the beginning and after activities, adjusting and ensuring adequate health surveillance of the workers. Although there was no reported health risk event in the studied WSP, its administration board has been developing preventive actions in addition to mandatory actions to comply with the legislation. Some examples of actions taken to prevent and mitigate the risk of occupational accidents are, for example, regular bioaerosol monitoring and the implementation of UV-C lamps in the cabin where workers are sorting waste.

In the context of the growing importance of the material recovery industry and the health preservation of its personnel, the aim of this study was to evaluate the indoor air quality in a Portuguese WSP, focusing on various physicochemical and microbiological parameters, such as particulate matter, carbon dioxide, relative humidity, temperature, bacteria, and fungi. The impact of waste-sorting activities on indoor air quality was assessed during different seasons and work hours. Additionally, the indoor air quality was assessed in administrative offices and canteen, and the effectiveness of UV-C air decontamination in the waste-sorting cabin was assessed.

2. Materials and Methods

2.1. Study Area

This study was conducted in two campaigns at the WSP located in the district of Porto, Portugal, which manages the domestic and industrial waste of about 1 million people. The first campaign took place from September 2021 to February 2022 and aimed to evaluate the occupational risk of workers who handle and sort various types of plastics in Waste-Sorting Unit 1 (WSU1), as shown in Figure 1. The waste comprises high-dimension plastics, plastic film, and other plastics collected from the municipal eco-centers and industry circuits. The materials are deposited in the unloading area (Figure 2a) and pre-screened by one to three workers. Then, another worker has the task of feeding a hopper with the waste that a conveyor belt/ramp will lift to the cabin (Figure 2b,c). The cabin is an elevated closed workplace with an area of 61 m^2, where waste is manually separated by 8 to 10 workers according to its composition: PVC, HDPE/LDPE, PP, and LDPE plastic films. These personnel use safety gloves, and their work shift is from 7 h to 15 h for 5 days/week. The cabin does not have any mechanical ventilation system. The exterior site (Ext1) is located about 30 m away from the building, as shown in Figure 1.

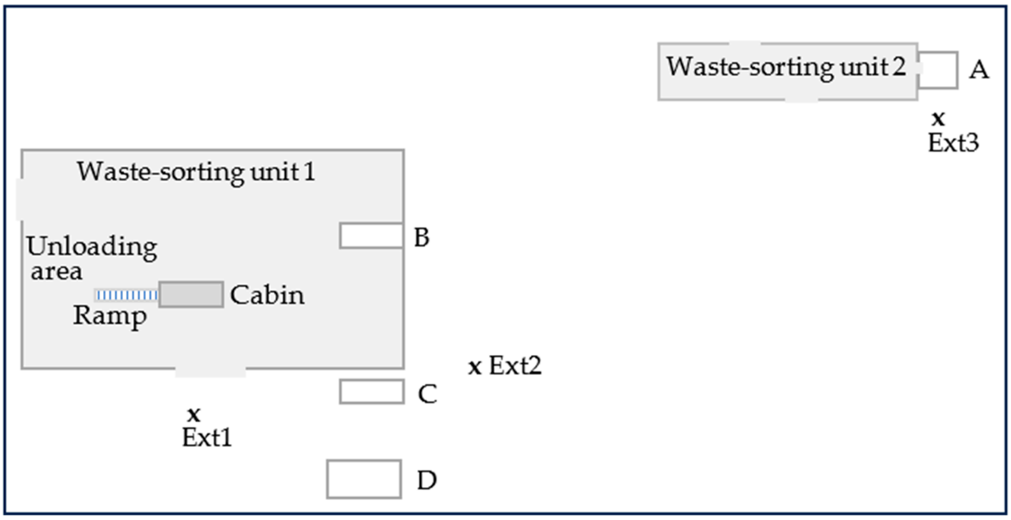

Figure 1. Scheme of the WSP. The administrative offices are marked by the letters "A", "B" and "C", and the canteen is marked with "D". Sites marked with "x" are the exterior sites.

Figure 2. Photographs of WSU1: (**a**) unloading waste area and the feed hopper; (**b**) the ramp; (**c**) the cabin where the UV-C lamps are indicated as L1 to L4.

A second campaign was carried out during September and November of 2023 with the objective of evaluating the occupational biological risk for the administrative staff working in the WSP. The characteristics of the analyzed administrative offices and the canteen are indicated in Table 1. Sites B, C, and D are close to WSU1, and Ext2 is the corresponding exterior air sampling site (Figure 1). The administrative location marked by A is about 310 m away, next to the Waste-Sorting Unit 2 (WSU2), which receives the plastic and metallic waste and cardboard packaging for liquid food from eco-points (Figure 1). All these locations are equipped with air ventilation systems. The exterior air sampling location corresponding to administrative office A is marked as Ext3 (Figure 1).

Table 1. Characteristics of the administrative offices and the canteen.

Sites	Volume (m^3)	Average Occupancy	Maximum Occupancy	Electrical Equipment and Ventilation
A—Administrative office	71	1	1	1 computer, 1 air conditioner
B—Administrative office	98	1–2	5	5 computers, 1 air conditioner with HEPA filters
C—Administrative office	173	5–8	22	22 computers, 4 air conditioners
D—Canteen	149	4–30	54	14 kitchen appliances, 6 air conditioners

The rationale for choosing the cabin and the location near the feed hopper (ramp) was to assess the health risk for the waste-sorting workers. In these cases, sampling was carried out before and during the workday to capture the variability in indoor air concentration. The administrative offices were chosen to assess the worst scenarios of pollutant contamination as they are close to the waste-sorting units and were analyzed in the morning and afternoon. The canteen was also selected because it is a place with a large influx of workers, especially at lunchtime, and may also be a place with increased contamination due to the activities of cooking/heating/washing food. Outdoor locations are reference points to assess the extent of contamination from indoor sources.

2.2. Sampling Methods

Microbiological and physicochemical parameters were measured monthly at preselected locations. Microbiological sampling was carried out with an impaction air sampler with a flow rate of 100 L/min (SAS Super ISO 100, VWR, Milano, Italy) on 90 mm plates with solid nutritive media. Sampled volumes ranged from 10 to 400 L, depending on the expected microbial concentration. At each location, three volumes were sampled in duplicate for each type of microorganism. The sampler head was cleaned with ethanol and dried before each sample collection. The sampler was placed at a height of about 1.5 m above the floor and at least 1 m away from the walls [48].

The physicochemical parameters measured in this study were particulate matter with a maximum diameter of 2.5 µm ($PM_{2.5}$) and 10 µm (PM_{10}) and the CO_2 concentration, temperature, and relative humidity. These parameters were determined with a CO_2 air quality monitor and particle measuring device BQ30 (Trotec, Heinsberg, Germany) with the following operating conditions: a resolution of 1 µm/m^3 for particulate matter; a resolution and accuracy of ±5% and 1 ppm for CO_2; a resolution and accuracy of ±2 °C and 0.1 °C for the temperature; a resolution and accuracy of ±3.5–5% and 0.1% for the relative humidity, respectively.

The effectiveness of UV-C disinfection of the air and surfaces of the sorting cabin was tested with the doors closed and no workers present. The test began one hour after the workday ended and was conducted as follows: the air was sampled on nutrient media plates, and duplicates of each plate were exposed to the light of the 4 UV-C lamps for 1 h. Additional air samples were collected to measure the effect on air quality, and all plates were incubated for further analysis.

2.3. Techniques for Bioaerosols Assessment

The bacterial bioaerosol concentrations were quantified on tryptic soy agar (TSA, VWR 84602) supplemented with cycloheximide (VWR 94271), which is a known inhibitor of fungi. Malt extract agar (MEA, VWR 84665) supplemented with chloramphenicol (VWR 0230EU), which inhibits the growth of a great majority of bacteria, was used to determine the fungi concentration [49,50]. The two-culture media were prepared according to the manufacturer's recommendations.

The plates were transported to the sampling locations and back to the laboratory after sampling in a thermal box at 4 °C and incubated in an inverted position under the following environmental conditions [50]:

- Mesophilic bacteria from environmental sources: 25 °C for 5 days;
- Bacteria from human sources: 37 °C for 2 days;
- Fungi: 25 °C for 5 days.

According to this European Standard, the incubation temperature for the mesophilic bacteria and fungi must be from 20 to 30 °C. However, other temperatures can be chosen as "the most appropriate temperature during cultivation near to that of the habitat in which the microorganisms were present before sampling" [50], p. 33. As humans are generally the most contaminating source of indoor air, the incubation temperature for bacteria is 37 °C in many studies found in the literature, which is also in accordance with the technical report from the Portuguese Agency for Energy [51]. In the present study, the bacterial counting was also assessed after incubation at 25 °C because the waste was outdoors for several days and exposed to similar temperatures in the warehouse prior to sorting.

Colonies were counted in plates containing 10 to 200 colonies for bacteria and 5 to 150 colonies for fungi [50]. The number of colonies was corrected according to the conversion table of the Most Probable Number of Colonies Forming Units (Pr) [52] due to the superposition of microbial particles impacting the same spot through the same sieve pore [48,53]. The bacteria and fungi concentrations were calculated using the methodology described in EN 13098:2019E [50] and according to the following Equation (1).

$$Concentration\left(\mathrm{CFUm}^{-3}\right) = \frac{\sum Pr_i}{\sum Volume_i} \tag{1}$$

The indoor microorganism concentration to the outdoor corresponding concentration ratio values (I/O) is a useful tool to assess the nature of the pollution exchange and the location of the highest emission sources. The I/O ratio was calculated by dividing the indoor concentrations by the corresponding outdoor concentrations. The I/O ratio was interpreted according to Neto and Siqueira [54], as cited in Stryjakowska-Sekulska et al. [55], in three grades: I/O $\leq$ 1.5 = good; I/O = 1.5 up to 2.0 = regular; and I/O > 2 = poor indoor ambient conditions.

The efficiency of bacterial decontamination with UV-C treatment was measured in the closed cabin in which four UV-C lamps (TUV 36W SLV/6, Koninklijke Philips N.V., Amsterdam, The Netherlands) emitting at 253.7 nm with 5.0 W irradiation power were in the ceiling along the conveyor belt. Several air samples were collected 1 h after the workers left. Some were sealed to function as reference controls, and others were placed open below each UV-C lamp. After 1 h of exposure, the plates were sealed, and more air samples were collected.

2.4. Quality Control

Quality control of culture media with antibiotics and sampling procedures is important to identify unwanted contaminations and guarantee the reliability of the findings [49,56]. The sterilization procedure was validated by the incubation of two uninoculated plates at 37 °C for 2 days and two uninoculated plates at room temperature for 3 days; none showed any contamination. During the study, it was observed that both media were suitable for bacteria or fungi growth, respectively, because of the observed colony characteristics. Even so, the residual growth of unwanted microorganisms was observed, especially if the plates were allowed to incubate for longer periods of time. According to EN13098:2019E [50], the addition of antibiotics is not mandatory, but it was necessary in the present study due to the excessive cross-contamination, especially in the medium for the bacteria.

The sampling was tested using blanks, which were culture media plates that underwent all procedures like the other plates but were not exposed to the air. In this study, no colony growth occurred in the blanks.

2.5. Statistical Analysis

The experimental data were analyzed using Microsoft Excel (version 2401) and IBM SPSS Statistics (version 28). The data were not all normally distributed according to the Shapiro–Wilk test, and thus, all the following tests were non-parametric. The Mann–Whitney test was used to compare the means of 2 samples, such as in samples collected in the cabin and near the ramp or in samples collected in the same location in the morning and afternoon. The Kruskal–Wallis test was used to compare the means of 3 or more samples, such as the samples collected across the workday time (3 different time periods) and sites (4 different sites). The significance values were adjusted by the Bonferroni correction for multiple tests. Spearman's rank correlation was used to assess the relationship between the physicochemical and microbiological parameters of the two campaigns. When performing two-sided tests, differences were considered statistically significant when the p value was lower than 0.05 unless otherwise stated.

3. Results and Discussion

3.1. Outdoor Air Quality

Samples of outdoor air were collected during both campaigns so their results could be compared with those for the indoor air. The physicochemical parameters of the outdoor air indicate a low CO_2 concentration (296–669 ppm), a temperature in the range of 11.0–26.2 °C, and a relative humidity in the range of 52.5–99.9%. The particulate matter $PM_{2.5}$ and PM_{10} were low in general, with medians of 7 and 13 µg/m^3, respectively, although they reached very high values, 689 and 913 µg/m^3, respectively, in the months of September and October 2021 (Table 2). It is probable that the elevated levels are a result of the ongoing highway construction in the vicinity.

Table 2. Physicochemical parameters of outdoor air.

	$PM_{2.5}$ (µg/m^3)	PM_{10} (µg/m^3)	CO_2 (ppm)	T (°C)	RH (%)
Mean	80	112	368	17.9	79.2
SD	180	240	55	5.5	14.4
Median	7	13	357	15.6	79.1
Min	1	2	296	11.0	52.5
Max	686	913	669	26.2	99.9

SD—standard deviation.

Particulate matter (PM) is composed of suspended particles with mineral and organic compounds, either in a liquid or solid state. The lower the dimension, the higher the probability of entering the respiratory system and compromising human health. The particles lower than 10 mm are the most deleterious as they are able to interfere with the gaseous exchanges in the pulmonary alveoli. Particulate matter concentrations in the outdoor air were within the same range as those in a city in Poland [57]; however, $PM_{2.5}$ and PM_{10} exceeded the protection threshold limits, 25 and 50 µg/m^3, respectively, in 21% of the samples, according to Portuguese regulations [36], although these are defined for commercial and services buildings. The values obtained for CO_2 outdoor levels were below the protection threshold of 1250 ppm, according to the same regulation.

The microbiological concentration of the outdoor air showed very high values for bacteria at 25 °C and fungi, with average values of 1654 and 1044 CFU/m^3, respectively (Table 3). The average concentration of bacteria at 37 °C had the lowest average value, 422 CFU/m^3, which is within the recommended threshold by the Portuguese legislation of 500 CFU/m^3, although this is for commercial and service buildings. These values are within those reported in the literature for the outdoor air. For example, Brągoszewska [27] obtained a similar outdoor air concentration of fungi (1221 CFU/m^3) but a higher concentration (1138 CFU/m^3) of bacteria at 37 °C in a WSP in Poland. Zuraimi et al. [58], in Singapore, observed higher outdoor concentrations of fungi aerosols near a childcare center

(1797.7–3559.8 CFU/m^3), and Z. Fang et al. [59] counted 71 to 22 100 CFU/m^3 for bacteria at 37 °C in Beijing urban areas (China).

Table 3. Microbiological parameters of outdoor air.

	Bacteria at 25 °C (CFU/m^3)	Bacteria at 37 °C (CFU/m^3)	Fungi (CFU/m^3)
Mean	1654	422	1044
SD	1150	347	497
Median	1568	338	1028
Min	333	48	425
Max	3208	1 150	1872

SD—standard deviation.

It is interesting to highlight that the bacteria incubated at 25 °C were about three-fold higher than those obtained at 37 °C, which could possibly be attributed to the fact that the outdoor air was at temperatures closer to 25 °C.

3.2. Indoor Air Quality in Waste-Sorting Unit 1 (Campaign 1)

3.2.1. Overall Indoor Air Quality Parameters

The overall concentration of the indoor air collected in the cabin presented higher values for the particulate matter $PM_{2.5}$ ($p < 0.05$) and CO_2 ($p < 0.001$) when compared to those near the ramp (Table 4). This may be because the cabin is a smaller and partially enclosed space where, normally, eight workers are manipulating and separating the waste.

Table 4. Physicochemical parameters of indoor air in the cabin and near the ramp.

	$PM_{2.5}$ (µg/m^3)	PM_{10} (µg/m^3)	CO_2 (ppm)	T (°C)	RH (%)
Cabin	22	42	491	16.6	83.1
Ramp	17	35	416	15.9	81.3
I/O Cabin	0.131	0.177	1.41	0.959	1.08
I/O Ramp	0.0990	0.149	1.19	0.920	1.05

The I/O particulate matter inside the cabin and near the ramp was very low compared to the other physicochemical parameters, but this does not directly indicate that the inside conditions were good. It appears that two sources of emissions may have been impacting the air quality, one internal and one external, with the latter having a greater impact. The outdoor air in the months of September and October had very high values of particulate matter that reached outdoor concentrations in the range of 284–686 and 379–913 µg/m^3 for $PM_{2.5}$ and PM_{10}, respectively. The CO_2 I/O ratio was above one, especially inside the cabin, which indicated the presence of an indoor CO_2 emission source. The temperature and the relative humidity had an I/O ratio close to one, thus indicating values similar to those found outdoors.

The concentration of microorganisms inside the cabin and near the ramp varied greatly and showed no statistically significant differences (Table 5). In some cases, the bacterial concentrations were indicated as lower limits of the real values since the lowest possible air samples contained too many colonies for accurate enumeration (>300 colonies). Even so, it is highlighted that, in general, the bacteria in the cabin presented higher average values than those from the ramp, while the opposite was verified for fungi. In both indoor sites, the concentrations were substantially higher than in the outdoor air, as observed by the I/O ratios above two, especially high for the bacteria at 25 °C, showing, as expected, that the activity of waste manipulation and revolving was an extensive pollutant emission source (Table 5). Similar values have been obtained by other authors; for example, in a study also carried out in a WSP in Portugal, total fungal concentrations were found to be in the order of 180 to 5280 CFU/m^3 [60]. Brągoszewska [27] reported airborne microbial concentrations in approximately similar ranges, namely 856–2711 CFU/m^3 for bacteria at 37 °C and 211–1237 CFU/m^3 for fungi at a WSP in Poland. Other authors have found more variable and higher concentrations in WSPs; for example, Lehtinen et al. [61] obtained concentrations ranging from 480 to 220,000 CFU/m^3 for bacteria at 25 °C and from 470 to 290,000 CFU/m^3 for fungi over a period of four seasons, and D.-U. Park et al. [3] reported 1.9×10^5 and 2.2×10^4 CFU/m^3 for bacteria at 37 °C and fungi, respectively.

Table 5. Concentration of bacteria and fungi in the air collected inside the cabin and near the ramp.

	Bacteria at 25 °C (CFU/m^3)	Bacteria at 37 °C (CFU/m^3)	Fungi (CFU/m^3)
Cabin	>21,470	2565	1488
Ramp	>14,684	>1397	2412
I/O Cabin	>14.56 *	3.96 *	1.44
I/O Ramp	>9.96 *	>2.16 *	2.34 *

* poor conditions (I/O > 2).

The waste management industry has been linked to health issues resulting from occupational exposure to airborne particulate matter and microorganisms, as reported by several authors over the past decades. For example, nine of a total of fifteen workers in a WSP in Denmark were diagnosed with occupational respiratory illness in the period from 1986 to 1988, and several of these continued to present symptoms two years after having left their jobs [62]. A study carried out in Denmark showed that waste-recycling workers are prone to skin, respiratory tract, upper airway mucosal, eye, and gastrointestinal problems, and the exposure of waste handlers to fungal spores, endotoxins, and β(1→3)-glucans at a sorting plant in Norway was reported to induce upper airway inflammation [24,63]. Wikuats et al. [13] observed that material-recycling workers self-reported higher percentages of respiratory, circulatory, gastrointestinal, and allergy symptoms when compared to control groups and that exposure to $PM_{2.5}$, PM_{10}, bacteria, and fungi may increase the possibility of symptoms such as coughing, phlegm, fatigue, malaise, itchy eyes or skin, and skin allergies. The potential of particulate matter and bioaerosol exposure to cause health problems in waste management workers has been evaluated through different strategies; for example, self-reporting through questionnaires, acoustic rhinometry, the analysis of the concentration of cells and proinflammatory cytokines in nasal lavage to access upper airway inflammation, the evaluation of the activation of toll-like receptors of the immune system, and cytotoxicity of settled dust against human epithelial lung cell lines, providing evidence for the occupational health risk for the workers in the waste management industry [15,24,64–66]. The reports on occupational exposure of waste collectors to bioaerosols have recently been reviewed in detail by Madsen et al. [26], who highlight that most studies are cross-sectional, and although they provide evidence of reduced lung function and inflammatory responses due to bioaerosol exposure, there is a need for longitudinal studies monitoring health effects to gain a better understanding of the long-term health effects of the occupation bioaerosol exposure of waste collectors.

It is interesting to highlight that the concentration of bacteria incubated at 25 °C was much higher than those obtained at 37 °C. As explained in the outdoor air, this could also be attributed to the fact that the temperatures at which the waste was exposed were closer to 25 °C.

3.2.2. Seasonal Effect

The physicochemical parameters of the indoor air in the cabin and ramp of the WSU1 (Figure 1) were evaluated during the autumn and winter of 2021. Although higher PM values were obtained for the cabin air, no significant differences were observed for this parameter for the two sites and seasons (Figure 3A,B). However, on average, the CO_2 and RH values were higher inside the cabin when compared to the ramp, and an increase in

CO_2 and RH alongside a temperature decrease was observed between autumn and winter (Figure 3C–E).

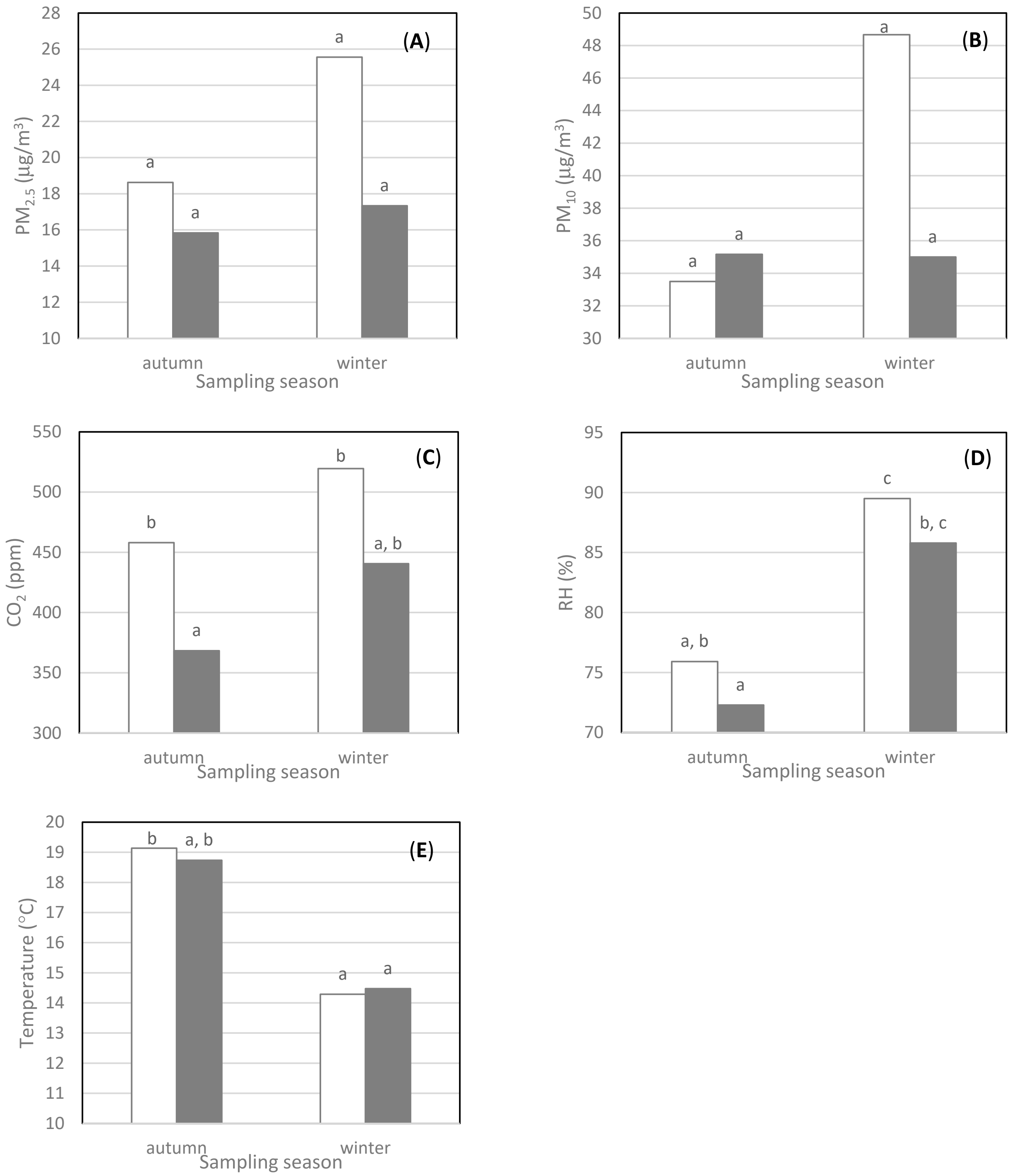

Figure 3. Physicochemical parameters of air collected inside the sorting cabin (white bars) and near the ramp (grey bars) in autumn and winter for (**A**) $PM_{2.5}$; (**B**) PM_{10}; (**C**) CO_2; (**D**) RH; (**E**) T. Different letters indicate a significant difference ($p < 0.05$) among the four bars in each figure.

The I/O ratios of the physicochemical parameters presented in Figure 3 were calculated to identify possible pollutant emissions in the indoor ambient (Table 6). The I/O ratio of particles in suspension inside the cabin and near the ramp in the autumn was very low.

However, this may not be an indication that the inside conditions relating to the particles in suspension were good, as explained in the previous section. The I/O ratios for CO_2 were above one, especially inside the cabin, indicating the presence of a moderate contamination source inside, which was probably more extensive in winter. The ratios for RH and T were close to one, as expected, from open areas to outdoor environments.

Table 6. Indoor to outdoor ratio relative to the physicochemical parameters of the air collected inside the sorting cabin and near the ramp in autumn and winter.

		I/O Ratio				
		$PM_{2.5}$	**PM_{10}**	**CO_2**	**T**	**RH**
Cabin	autumn	0.0578	0.0764	1.36	0.90	1.16
	winter	1.45	1.52	1.44	1.08	1.01
Ramp	autumn	0.0491	0.0802	1.09	0.880	1.11
	winter	0.981	1.09	1.22	1.09	0.964

The concentration of bacteria and fungi in the cabin and ramp during autumn and winter did not show significant differences; however, there is a clear tendency for higher values during winter (Table 7). The values obtained in the cabin and the ramp for each season showed no significant variations, probably due to the fact that the air-contaminating activity of revolving the waste was occurring in both sites: manually inside the cabin and near the ramp, by way of the feed hopper and grab loader and the waste moving upwards on the ramp.

Table 7. Concentration and I/O ratio of bacteria and fungi in the air collected inside the cabin and near to the ramp.

	Bacteria at 25 °C (CFU/m^3)		**Bacteria at 37 °C (CFU/m^3)**		**Fungi (CFU/m^3)**	
	Autumn	**Winter**	**Autumn**	**Winter**	**Autumn**	**Winter**
Cabin	1475	26,469	408	3021	675	2300
Ramp	5567	16,964	>2650	1084	2025	2800
I/O Cabin	0.681	20.3 *	0.584	4.61 *	1.51	1.73
I/O Ramp	2.57 *	13.0 *	>3.80 *	1.65	4.54 *	2.10 *

* poor conditions (I/O > 2).

In some cases, the I/O ratios for both sites and both seasons were above two, a clear indication of the poor air conditions inside compared to the outside air. This tendency was aggravated in the winter, especially for bacteria at 25 °C, when there was a significant increase in RH. A significant relationship between humidity and bacterial and fungal exposure during waste-sorting activities has also been reported by D.-U. Park et al. [3].

Seasonal effects on air microbial concentrations and diversity at waste-sorting centers and other indoor environments have previously been reported by other authors [9,30,57]. Very variable concentrations for bacteria and fungi were reported in two WSPs over four seasons [61]. On the other hand, a comparison of the air quality in a fully automated WSP and a manual WSP revealed significant differences in occupational exposure, which was also dependent on the tasks performed [15]. Therefore, occupational exposure to the bioaerosol concentration and its composition and viability is dependent on several factors, such as the type of waste material, installation, performed tasks, and factors such as relative humidity, temperature, and radiation exposure, among others. Additionally, it is challenging to compare the results of the available studies due to various sampling methods and sample processing.

3.2.3. Workday Time Effect

The indoor air quality parameters were obtained for samples collected at different times of the workday during winter and inside the cabin and ramp as follows: 1 h before and 1 h after the workday (6 h and 8 h) and 1 h before the workday ends (14 h). For each parameter, significant differences were verified only between the highest and the lowest values (Figure 4).

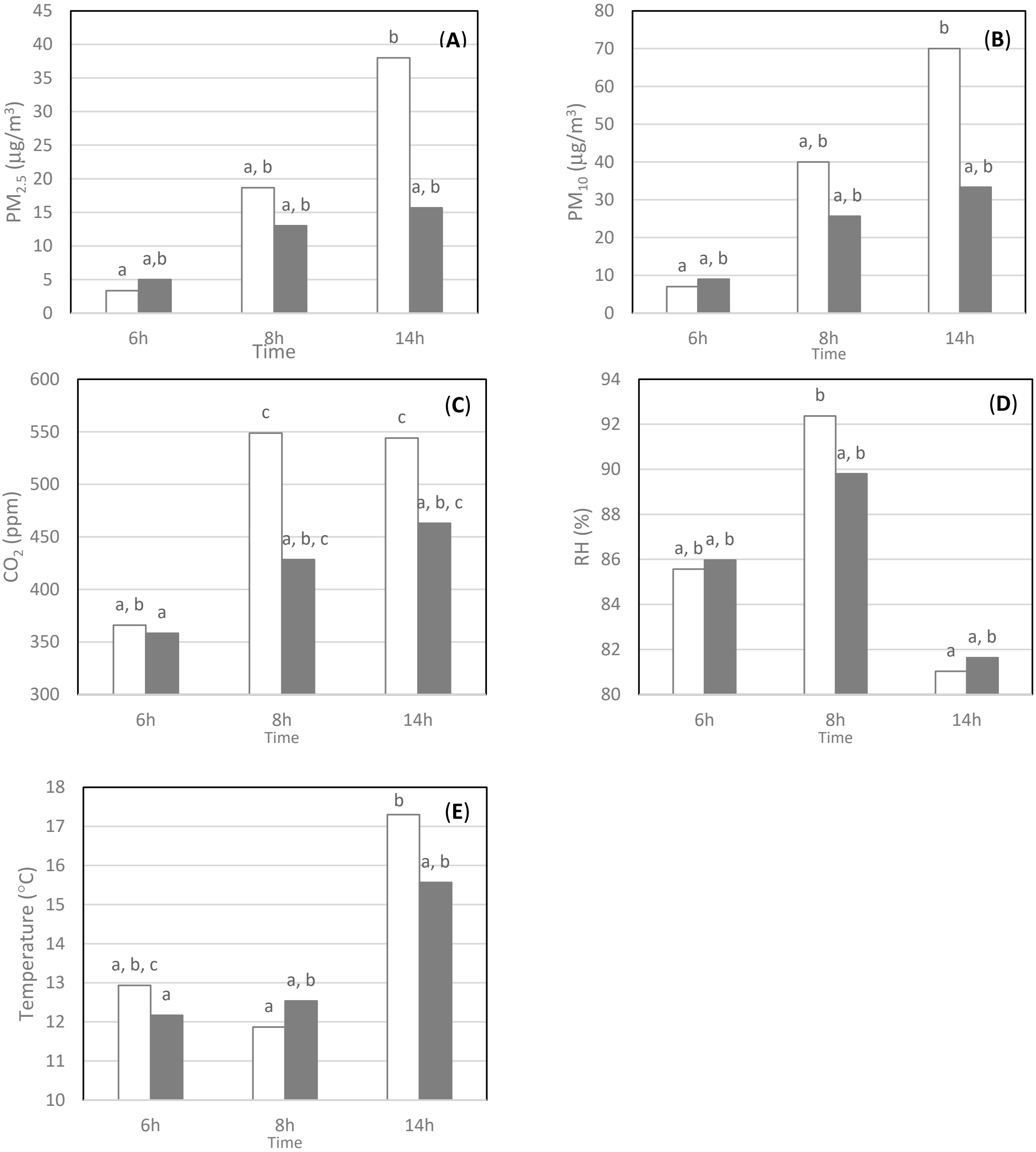

Figure 4. Physicochemical parameters of air collected inside the sorting cabin (white bars) and near the ramp (grey bars) during the workday for (**A**) $PM_{2.5}$; (**B**) PM_{10}; (**C**) CO_2; (**D**) RH; (**E**) T. Different letters indicate a significant difference ($p < 0.05$) among the four bars in each figure.

In general, the concentration of particulate matter increased during the workday. The highest values were obtained for the cabin, reaching 38 μg/m^3 for $PM_{2.5}$ and 70 μg/m^3 for PM_{10}, which are higher than the recommended thresholds for commercial and service buildings [36]. A similar trend was observed for the carbon dioxide concentration; however, the maximum values were around 550 ppm, which falls within the range of good air quality (Figure 4). The temperature and relative humidity showed an inverse trend from 8 h to 14 h, i.e., when T increased, RH decreased. This fact does not necessarily indicate that moisture was removed from the air; the capacity of the air to hold moisture increased, making it less concentrated and decreasing its relative percentage. It is important to note that the relative humidity was consistently higher than 80%, while the optimum working conditions indicated in Decree-Law no 243/86 [67], are in the range of 50 to 70% for offices and commercial and service establishments. In addition, the temperatures in WSU1 were also below the recommended range, which is 18–22 °C.

The concentration of bacteria was higher inside the cabin than near the ramp during working hours (Table 8). Comparing the values during the workday for both sites, the lowest was at 6 h, one hour before the beginning of the work shift, then the numbers increased significantly at 8 h, followed by a decrease at 14 h. This temporal trend may be attributed to waste-sorting activities, temperature increases, and humidity decreases. However, some exceptions to this trend were observed, probably due to the high variability. For example, in samples collected in November of 2021, the concentration of microorganisms was higher near the ramp than cabin values, and in December of 2021, the fungi concentrations were higher than those of bacteria at 25 °C. The I/O ratios indicate poor conditions (I/O > 2), especially one hour after the work shift, 8 h, as can be observed in Table 8.

Table 8. Concentration and I/O ratio of bacteria in the air collected inside the cabin and near the ramp.

	Bacteria at 25 °C (CFU/m^3)			Bacteria at 37 °C (CFU/m^3)		
	6 h	**8 h**	**14 h**	**6 h**	**8 h**	**14 h**
Cabin	1540	>53,000	24,000	108	3567	1740
Ramp	3395	>53,000	10,700	47	2533	698
I/O Cabin	0.604	>16.52 *	24.74 *	0.806	7.46 *	5.16 *
I/O Ramp	1.33	>16.52 *	11.03 *	0.351	5.30 *	2.07 *

* poor conditions (I/O > 2).

The sharp increase in the bacterial concentration within the first hour of the work shift suggests that work activities are a significant contributing factor. Human presence and activity have also been found to influence indoor bacterial content [68]. This is also corroborated by the high I/O ratios for the cabin and ramp. Other authors have observed increases in airborne microorganisms, such as fungi, during the work shift in plastic waste-sorting facilities [9]. Stryjakowska-Sekulska et al. [55] conducted a study on the concentration and diurnal variation in microorganisms in various university rooms, such as classrooms, laboratories, libraries, canteens, office spaces, and bathrooms, and reported that during daily activities, bacterial concentrations increased up to 8-fold, except in a ventilated lecture room. The high I/O ratios obtained in this study also suggest poor ventilation of the workspaces (Table 8). An interesting detail of the bacterial concentration was the decrease towards the end of the work shift in the cabin and at the ramp. This may have been due to the type of activities and a possible reduction in the work intensity towards the end of the work shift, and it may also be related to a decrease in the relative humidity (Table 8). On the other hand, a study of diurnal variation in outdoor bacterial concentrations carried out in Beijing reports a significant decrease at 13 h compared to 9 and 17 h; the authors suggest that this might be due to a reduction in human activity and traffic and an increase in temperature and UV radiation [59]. In this particular case, a decrease in the outdoor bacterial concentrations was observed, accompanied by a significant reduction in the indoor concentration resulting, in a decrease in most I/O ratios between 8 h and 14 h.

3.3. Indoor Air Quality in Administrative Offices and Canteen (Campaign 2)

Three administrative offices and the canteen of the WSP were selected to follow the physicochemical and microbiological parameters during the workday, and samples were collected in the morning and afternoon. The average daily values for some of the physicochemical parameters, namely $PM_{2.5}$, PM_{10}, and CO_2, varied significantly ($p < 0.001$) among the different sites (Table 9). At site D, the canteen, $PM_{2.5}$ (16 μg/m^3), and PM_{10} (34 μg/m^3) were different from the corresponding values found in B and C, probably due to the higher occupancy and continuous operating kitchen appliances (refrigerators, fridge, microwave ovens, dishwasher, etc.). Related to CO_2, all comparisons showed different values in each site, except for when comparing D (826 ppm) with B (1194 ppm) and B with C (1338 ppm).

Table 9. Averaged daily concentration and I/O ratio of physicochemical in the air collected in sites A, B, C and D.

	$PM_{2.5}$ (μg/m^3)	**PM_{10} (μg/m^3)**	**CO_2 (ppm)**	**T (°C)**	**RH (%)**
Site A	90	118	561	19.4	81.5
Site B	4	8	1194	19.3	81.8
Site C	6	11	1338	19.3	82.1
Site D	16	34	826	21.8	78.4
I/O site A	17.18 *	12.4 *	1.38	1.03	0.954
I/O site B	0.731	0.753	3.22 *	0.915	1.13
I/O site C	0.917	1.08	3.61 *	0.92	1.13
I/O site D	2.74 *	3.20 *	2.23 *	1.04	1.08

* poor conditions (I/O > 2).

The I/O ratios for a CO_2 concentration higher than two showed that locations B, C, and D presented poor indoor environmental conditions (Table 9), which may have corresponded to an extensive indoor pollutant emission or insufficient ventilation rate. The particulate matter concentration also reached high values in samples collected in locations D and A in particular. There were no significant variations in the temperature and relative humidity when compared to the outdoor values.

The bacterial concentrations were similar for all locations, averaging from 295 to 464 CFU/m^3. The fungal concentrations increased in the order of A < B < C < D (Table 10). Site D had the highest concentration of fungi, possibly due to high occupancy and the presence of kitchen appliances. In most cases, the I/O ratios showed similar microbial concentrations to the outdoors, except for bacteria in office A (I/O > 2) and fungi in the canteen (I/O $\rightarrow$ 2) (Table 10).

The microbial concentration in the administrative offices and canteen was significantly lower than those obtained for the ramp and cabin of the sorting area. A study of the exposure to inhalable dust, endotoxins, and microorganisms of Danish domestic waste-recycling industry workers found higher levels of exposure for the production workers than the administrative workers [69]. Additionally, significant differences in microbial exposure between employees working in the production area and those working in offices were also reported for biowaste pretreatment plants [70]. A study on the culturable bioaerosol in four types of buildings (residential, schools, offices, and hospital) in China reported similar bacterial and fungal concentrations as the ones obtained in this study, namely, 203.8 ± 188 CFU/m^3 and 352.5 ± 476 CFU/m^3, respectively, in Winter [71]. The concentration of bacteria and fungi has been reported to vary due to room occupancy, ventilation, and the type of activities that are carried out [55]. It was also observed that a proper ventilation system in a lecture room was able to keep the I/O ratios close to one, thus maintaining good indoor air quality [55].

Table 10. Concentration of bacteria from human sources and fungi in the air collected in the offices and canteen.

	Bacteria at 37 °C (CFU/m^3)	**Fungi (CFU/m^3)**
Site A	295	373
Site B	464	723
Site C	424	851
Site D	370	1604
I/O site A	2.33 *	0.307
I/O site B	1.21	0.848
I/O site C	1.10	1.00
I/O site D	0.964	1.88

* poor conditions (I/O > 2).

3.4. Physicochemical and Microbiological Parameter Correlations

The correlations between the parameters were considered acceptable for their absolute values if they were higher than 0.3 [72,73] and were classified as having a low (0.30–0.50), moderate (0.50–0.70), high (0.70–0.90), or very high (0.90–1.00) correlation.

Among the physicochemical parameters in the first campaign, significant correlations were found between $PM_{2.5}$ and PM_{10} (very high direct correlation), consistent with the data reported by Baghani et al. [19], and the temperature and humidity (moderate inverse correlation) (Table 11). The decrease in relative humidity may be related to temperature increases, as this will induce a higher moisture capacity. Moderate direct correlations were found between bacteria and CO_2 concentrations, indicating poor ventilation. A high positive correlation was observed between the fungi concentration and relative humidity, as observed by other authors [18].

Table 11. Physicochemical and microbiological parameter correlations for campaign 1.

	1	**2**	**3**	**4**	**5**	**6**	**7**
1. $PM_{2.5}$	--						
2. PM_{10}	0.980 *						
3. CO_2	−0.091	−0.125					
4. RH	0.199	0.161	0.429				
5. T	0.309	0.350	0.007	−0.675 *			
6. Bact. at 25 °C	0.017	0.033	0.683 **	−0.033	0.383		
7. Bact. at 37 °C	0.232	0.200	0.236	0.355	−0.036	0.633 ***	
8. Fungi	0.204	0.096	0.359	0.731 **	−0.587	0.314	0.299

Correlation highlighted in grey is significant at the level of * 0.01, ** 0.05 and *** 0.10.

Although correlations of the microbial concentrations with environmental parameters were identified, controlling them to favor air quality is not a practical intervention within the sorting areas, which are partially open spaces that allow waste flux. In addition to the current practices in the studied WSP, like enhanced natural ventilation and daily cleaning of the sorting cabin and workers' clothes, there are still some effective measures that can be implemented. These include the use of protective equipment by workers and the daily decontamination treatment of indoor air and surfaces in the cabin using UV-C or germicides.

During campaign two, significant correlations were found between $PM_{2.5}$ and PM_{10} (very high direct relation) and temperature and humidity (low inverse relation), which is similar to campaign one (Table 12). The bacterial concentration exhibited a low correlation with the temperature and CO_2 concentration, which suggests an insufficient ventilation rate of the compartments. Interestingly, other authors have reported an inverse relationship between the temperature and bacterial concentrations, which are accompanied by direct correlations with the relative humidity [8,19]. On the other hand, the fungi concentration was found to be directly correlated to $PM_{2.5}$, PM_{10}, CO_2, bacteria, and employee presence. A correlation between the PM and the concentration of fungi was also reported for other indoor environments, such as schools [74]. Particulate matter has a complex relationship with fungi, which is influenced by the PM size, composition, environmental conditions, and human activities.

Table 12. Physicochemical and microbiological parameter correlations for campaign 2.

	1	2	3	4	5	6	7
1. $PM_{2.5}$	--						
2. PM_{10}	0.986 *						
3. CO_2	0.112	0.165					
4. RH	−0.114	−0.157	0.213				
5. T	0.052	0.126	0.058	−0.399 ***			
6. Bacteria at 37 °C	0.190	0.240	0.361 ***	−0.252	0.495 **		
7. Fungi	0.482 **	0.510 **	0.446 **	0.150	0.292	0.491 **	
8. Workers number	0.185	0.192	0.696 *	0.292	0.065	0.305	0.512 **

Correlation highlighted in grey is significant at the level of * 0.01, ** 0.05 and *** 0.10.

Based on the correlations identified between the microbial composition and the physicochemical parameters within the administrative offices and canteen, it would be beneficial to take certain measures to enhance indoor air quality. Regular maintenance of air conditioners and the implementation of an online monitoring system can help ensure that environmental parameters, such as PM, T, RH, and CO_2, remain within the recommended ranges. Additionally, it is important to substitute furniture and building materials if they emit volatile organic compounds and to consider introducing potted plants or green walls to help purify the air [75,76].

The mitigation of pollutant contamination in the surrounding outdoor air could also be crucial for decreasing the impact on nearby communities. This may be accomplished by covering the outside waste storage prior to sampling and creating a green buffer zone surrounding the buildings with trees, bushes, and other vegetation, having the latter already been implemented. The promotion of workers' training is also an important measure to foster a culture of environmental awareness and responsibility [35,76].

3.5. UV Disinfection Effect on Indoor Air Quality

The UV disinfection effect on the air and surfaces was tested with a system of 4 UV-C lamps in the cabin after the work shift. The results show the UV-C treatment efficiently disinfected the air and surfaces, with disinfection efficiencies of at least 87.6% for the bacteria (Table 13). The particulate matter ($PM_{2.5}$ and PM_{10}) was also measured before and after the UV-C treatment and decreased by about 50%.

Table 13. Bacteria concentration before and after UV exposure for 1 h inside the cabin and disinfection efficiency.

	Concentration (CFU/m^3)		Disinfection Efficiency (%)
	BEFORE	**AFTER**	
Bacteria at 25 °C	11,875	Surface: 421	96.5
Bacteria at 37 °C	464	Surface: 58 Air: 47	87.6 89.9

The use of UVGI to decontaminate hospital environments has a long history and has recently regained interest [37,46,77]. Various strategies may be employed for air disinfection; for example, whole-room irradiation if the space is unoccupied, upper-room irradiation of occupied rooms, or in-duct UVGI in ventilation systems [37]. For example, Li et al. [78] tested an industrial-grade cleaner prototype combining filtration and UV-C light on the long-term improvement of air quality and found a 55% reduction in PM and a 47%

reduction in airborne viable bacteria between the days the device was off and then turned on. When employing UVGI for air and surface disinfection, it is important to take into consideration the effect of distance, shadows, and dirt, which reduce its efficiency [46,77,79]. Additionally, it has been found that microbes have a higher resistance to UVGI at high relative humidity levels [37]. There may also be concerns about the safety of human operators as UVGI can cause erythema and photokeratitis; however, these can be prevented by correct use, the maintenance of upper-room UVGI, and the use of far-UVC in the range of 200–222 nm, which have shown promise to be effective against bacteria without skin-damaging effects [37,80]. On the other hand, UVGI may also impact atmospheric chemistry, such as increases in PM and gas-phase compounds during high-intensity irradiation and the production of ozone by some short-wave lamps [81,82]. Therefore, our results suggest that UVGI could be used as a potential method to reduce biological risk in waste-sorting facilities and protect the health of workers, provided that the aforementioned factors for the proper and safe application are taken into account.

4. Conclusions

The purpose of this study was to assess the indoor air quality in a WSP in Portugal, focusing on various physicochemical and microbiological parameters, such as particulate matter, carbon dioxide, relative humidity, temperature, bacteria, and fungi. Overall, waste-sorting activities were found to negatively affect the quality of indoor air, particularly in terms of microbial concentrations and particulate matter, thus exposing the employees to an increased health risk. Furthermore, air quality worsened in the winter season as compared to autumn, particularly with respect to bacteria at 25 °C and fungi concentrations. During the work shift, the concentration of particulate matter increased, as expected, along with the concentration of microorganisms in the air. However, towards the end of the shift, the microbial concentration decreased, while the temperature and relative humidity showed an inverse correlation. It is apparent that several factors affected the presence and variation in airborne microorganisms in the waste-sorting area. Nevertheless, the high concentration of these microorganisms poses a significant health risk to employees, and therefore, it is vital to implement measures to mitigate this risk. The application of UVGI shows potential for this mitigation.

This study also raises the question of whether the optimal concentration to incubate the sampled bacteria should be 37 °C, as performed in the major studies found in the literature, and if the analysis should also be carried out at 25 °C to complement the findings.

The working environments of other WSP employees, such as administrative workers, were also compromised, although they were not directly involved in waste-sorting activities. In these areas, occupancy was one the factors that mostly affected the air quality, particularly for CO_2, relative humidity, and fungi, which surpass the health thresholds and indicate poor ventilation and air circulation in these spaces.

This study has important implications for indoor air quality in WSPs. It contributes to the understanding of working conditions not only in the waste-sorting areas but also in administrative areas and other facilities within the same organization. Based on this information, the scenarios that pose the highest risk to workers' health can be identified, and practical and targeted measures can be designed to enhance indoor air quality.

Some limitations in this study can compromise the generalization of the findings to other contexts and regions. First, sampling was carried out in a WSP that treated a mixture of uncontrolled waste materials within a Mediterranean climate, which is different from other regions. Furthermore, the lack of control over these conditions compromises the assessment of environmental factors. Secondly, the measurement of bioaerosols based on culturable techniques is likely to exclude a high percentage of those that are viable or unable to form colonies on the nutrient media plates [49,83]. For example, Harkawy et al. [84] observed that in a naturally ventilated library building, less than 3.9% of the total microorganisms were culturable. Manuel et al. [85] also observed that the environmental conditions (temperature and nutrient availability) highly affected the fraction of culturable and viable bacteria in drinking water distribution systems.

There are still some questions that remain unanswered after the present work. Although the concentration of bioaerosols may be an indicator of indoor microbial contamination, the identification of microorganisms by gene sequencing would be essential to complement the present findings. Moreover, this technique can also distinguish between the composition of culturable microorganisms at 25 or 37 °C, identifying an adequate incubation temperature for samples from WSPs. Total and viable microorganism concentrations can be evaluated by epifluorescence microscopy, providing further information on bioaerosol compositions. Following the fact the UV-C treatment proved to be an effective decontamination method in WSPs, further tests should be performed to optimize the operational conditions and assess its long-term effect on indoor air quality.

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