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AIRBORNE MICROORGANISMS ASSOCIATED WITH PACKAGING GLASS SORTING FACILITIES

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In recent years, efforts have been undertaken to reduce the volume of residual waste through sorting and recycling. The waste management and recycling sector is thriving and the number of workers there is increasing. In this context, prior knowledge of the risks to which workers may be exposed is of crucial importance, and preventive measures need to be put in place to accurately identify and quantify those risks. This study aimed to assess occupational risk of exposure to biological agents (viable bacteria and fungi) in a Portuguese waste packaging glass sorting plant. Air samples were collected from selected locations in waste sorting cabins (critical area, CA), administrative services (noncritical area, NCA) and outdoors (control point, CP). Duplicate air samples were collected through an impaction method. The investigation was carried out over an 8-mo period with two collection periods, autumn/winter (AW) and spring/summer (SS), in order to access the influence of any seasonal variation. In the 36 air samples collected, 319 bacterial and 196 mold identifications were performed. Air samples revealed existence of high environmental contamination by bacteria (1.6×10^4 colony forming units [cfu]/m^3) and fungi (1.5×10^4 cfu/m^3). The predominant bacterial genus was *Staphylococcus* (coagulase negative) with values ranging from 29.6 to 60% of the total count of bacteria. Genera *Bacillus, Micrococcus*, and *Staphylococcus* (coagulase negative) were also present at all sampling sites, regardless of the season. However, the counts of these genera, in the CA, were higher in warmer seasons. The genus *Penicillium* was the most frequent genus present with an approximate value of 95% of total fungal count in the CA. Seasonal variation was a significant factor for total bacteria and fungi, except for NCA versus CP. Overall, the highest levels of bacterial and fungal species (10^4 cfu/m^3) were found in the waste sorting cabin (CA). These results highlight the importance of proper design and risk evaluation when planning a new waste facility, such that working conditions minimize proliferation of biological agents in the workplace.

New industrial activities have emerged in recent years where exposure to biological agents is of relevance. The waste management and recycling sector is thriving and the number of workers is increasing sharply. The International Labour Organization (ILO) green jobs program identified waste management as one of the most rapidly growing sources of green employment (European Agency for Safety and Health at Work, 2013; United Nations Environment Programme, International Labour Organization, International Organization of Employers, and International Trade Union Confederation, 2008). The "Expert Forecast

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on Emerging Biological Risks Related to Occupational Safety and Health" of the European Agency for Safety and Health at Work proposed that the introduction of new treatment technologies and manual sorting brought new risks to workers involved in collecting, sorting, recovery, and final disposal of waste (European Agency for Safety and Health at Work, 2007). Further, existing regulations directed at waste management were only considered from an environmental perspective, not taking into account any aspects related to health and safety at work.

In particular, during the operations for waste sorting, professionals deal with preselected waste. Although disposal of hazardous materials is prohibited, it is difficult to always comply with regulations, whether intentionally or inadvertently, and thus some of these dangerous materials are deposited, creating special problems and increased health risks to workers. The use of packaging glass for the glass industry is only possible if there is a careful routine for selecting and removing undesirable materials. Thus, workers involved in the selection of such materials are subjected to various occupational hazards, especially to exposure to biological agents.

Several studies focused on the topic of environmental contamination by bacteria, fungi, and their metabolites, trying to establish a link between occupational exposure and occurrence of specific symptoms among employees, attributed to their exposure to such elements (Sabino et al., 2012; May et al., 2012; Thomas, 2013; Dutkiewicz, 1997). Currently, there is clear evidence correlating work activities involved in the collection, transport, treatment, recovery, and disposal of waste and the appearance of specific symptoms among workers. The main symptoms are related to gastrointestinal, respiratory, inflammatory, and skin problems, organic toxic syndrome (ODTS), and irritation of the eyes and mucous membranes (Bünger et al., 2000; Gladding et al., 2003a; Heldal et al., 2003; Herr et al., 2004; Marth et al., 1997; Poulsen et al., 1995a, 1995b; Von Essen et al., 1990; Würtz and Breum, 1997; Domingo and Nadal, 2009).

The term "waste recycling worker syndrome" was suggested for flu-like symptoms, airway irritation, and inflammation of the eyes often observed in employees of the waste industry (Gladding et al., 2003b; Gladding, 2002; Poulsen et al., 1995b). Although exposure to bioaerosols in the waste industry was already focused on several studies (Gladding and Coggins, 1997; Kiviranta et al., 1999; Krajewski et al., 2002; Malta-Vacas et al., 2012; Park et al., 2011; 2013; Solans et al., 2007; Viegas et al., 2014b), to our knowledge, the assessment of occupational exposure to biological agents in the packaging glass recycling industry, with particular emphasis on activities of sorting glass, has not yet been performed. It is of interest that Kennedy et al. (2004) assessed the impact of a point-of-sale glass bottle recycling on indoor air quality and employee health. The aims of the present study were to characterize the viable microbial community (bacteria and fungi) in a packaging glass waste sorting facility and to determine the influence of seasonal variation.

METHODS

Examined Facilities

Air sampling was performed in one of the two existing Portuguese waste packaging glass sorting plant with 25 employees. The plant receives 183,000 tonnes of packaging glass per year, selectively collected by municipalities, which represents part of the annual production of Portuguese domestic glass packaging waste. The glass is discharged into the raw material park, where it remains until it is forwarded to the screening process. Glass is then transported by a loader into the feed bin of the plant, moving along the conveyor belt to the sorting compartment. The process includes several manual and automatic tasks for sorting the hull, with the ultimate goal of removing any existing contaminants (paper, plastic, metal, porcelain).

This study was carried out during an 8-mo period covering two collection periods:

autumn/winter (AW) and spring/summer (SS). Sampling was performed between November 2010 and June 2011. Air samples were collected at different working locations in the plant:

- A critical area (CA), where the workers sort the glass and are therefore directly exposed to the glass waste. The sorting cabin is 5 m^2 and the air-conditioning system does not permit air renovation, but only carries out temperature regulation. Within the waste sorting plant, the selected sampling site was the one that presented the worst conditions regarding structural conditions. The employees involved in waste sorting rotated between work tasks in the sorting facility that operates 24 h/d, 7 d/wk, on a daily regimen of three 8-h shifts. Workers did not use respiratory protection devices. At this point six air samples were collected in the SS season and seven in the AW season.
- A noncritical area (NCA), where workers are not directly exposed to the process of glass waste screening. In this particular plant, the administrative area is located in the same building as the glass sorting cabin and features natural ventilation and an air conditioning system. At this point six air samples were collected in the SS season and six in the AW season.
- A control point (CP), which in this case is an outdoor reference sample. Since the glass is stored outside, air samples were collected outside the plant facility. At this point five air samples were collected in the SS season and 6 in the AW season.

Sampling

Thirty-six duplicate air samples were collected at the different working locations at a height of 1 m, using a stationary Sampl'air Lite one-stage impactor from AES Chemunex (Bruz, France). The volumes of air sampled were in accordance with the estimated existing microbial contamination at each sampling point. In the CA the air volume was 50 L, whereas in the NCA and CP the air volume was 100 L at a flow rate of 20 L/min. Calibration of the sampler was performed by an external accredited organization, according to ISO 17025:2005. The sample plan in relation to number of samples was performed in accordance with the American Conference of Governmental Industrial Hygienists (ACGIH) recommendations (ACGIH, 1999).

Microbiological Analyses

Airborne microorganisms were collected on specific sampling media plates for bacteria (nutrient agar agar) and fungi (malt extract agar) supplemented with chloramphenicol (0.05%). Petri plates were incubated aerobically at 37°C for 48 h and at 25°C for 3 to 5 d, for bacteria and fungal counts, respectively. According to the amount of sample collected and the selected method, the limit of detection (LOD) (20 cfu/m^3) and maximum limit of quantification (LOQ) (25,180 cfu/m^3) were considered.

Pure cultures obtained from single colonies were subjected to presumptive identification by morphologic observation and biochemical testing, as recommended by *Bergey's Manual* (Krieg, 1984; Sneath et al., 1986; Williams and Sharpe, 1989). In addition, selected isolated colonies were identified using microtests API 50 CH (Biomérieux, Marcy l´Etoile, France), API 50CHB/E (Biomérieux, Marcy l´Etoile, France), API *Coryne* (Biomérieux, Marcy l´Etoile, France), API 20 *Strep* (Biomérieux, Marcy l´Etoile, France), BBL Crystal Enteric/Nonfermenter ID kit (BD Diagnostics Systems Europe, Sparks, MD), and BBL Crystal Gram-Positive ID kit (BD Diagnostics Systems Europe, Sparks, MD). API 32C identification ID (Biomérieux, Marcy l´Etoile, France) was used for the phenotypic identification of yeast cells. Inoculation of a plate with chromogenic culture medium BBL CHROMagar Candida (BD Diagnostics Systems Europe, Sparks, MD) was used to assess the existence of strains of *Candida albicans* and *Candida tropicalis*. After isolation in malt agar, filamentous fungi were identified according to their macroscopic morphology, and microscopic observation of the vegetative (mycelia)

and reproductive structures of the fungi took place.

Statistical Analysis

For statistical analysis of data, the IBM SPSS Statistics programme 19.0 (SPSS, Chicago, IL) was used. Since data were not normally distributed (Kolmogorov–Smirnov test of normality with Lilliefor's significance correction), the non-parametric Kruskal–Wallis test, followed by a one-way analysis of variance (ANOVA) with the LSD Fisher test ($\alpha = .05$), was used.

RESULTS

The mean counts of total bacteria and fungi in the CA (1.6×10^4 cfu/m^3 and 1.5×10^4 cfu/m^3, respectively) were always higher than those obtained at the CP (5.2×10^2 cfu/m^3 and 1.3×10^3 cfu/m^3, respectively) and NCA (1.2×10^3 cfu/m^3 and 1.6×10^3 cfu/m^3, respectively). The multiple comparisons between the three sampling points for total bacteria counts, using the LSD test, presented significant differences. For total fungi counts, significant differences were found, except between the NCA and CP.

Seasonal Variation

For the three sampling points, the warmer periods of the year (SS) displayed higher mean counts compared to cooler periods (AW), with CA being the most contaminated spot (Tables 1 and 2). Further, total counts of fungi for SS were higher in CP than in NCA (Table 2). In all seasons, there is clearly more contamination in CA than in NCA and CP. In addition, for both total bacteria and total fungi, it was noted that in NCA and CP the dispersion of values was lower than in CA (Figure 1). In order to verify whether there are significant differences between areas/seasons, both for total bacteria and fungi, the Kruskal–Wallis test was applied, which confirmed that significant differences existed. For total bacteria, season was not a significant factor at each sampling point (Table 3). For total bacteria and fungi, all differences for both areas/seasons were significant, except for the areas/seasons represented in Table 3.

Microbiological Characterization of Environmental Samples

As a result of the 36 air samples collected in the packaging glass industry, 319 bacterial identifications were performed. The predominant genus was *Staphylococcus* spp. (coagulase negative) with values ranging from 29 to 60% of total bacteria count at the three sampling points. However, at CA in the AW season the *Staphylococcus* spp. represented only 5.2%, whereas *Serratia* spp. with 39.6% was the dominant genus. Table 1 illustrates the mean total bacterial counts of each genus identified, by season and sampling point.

The genera *Bacillus, Micrococcus*, and *Staphylococcus* (coagulase negative) were present at all sampling points, regardless of the season. However, the counts of these genera, in CA, were higher in the SS season. In CA, the genera *Proteus, Enterobacter, Klebsiella, Pseudomonas*, and *Corynebacterium* were identified belonging to group 2 of the classification of biological agents (European Parliament, 2000). The genera *Corynebacterium* and *Proteus* exhibited mean counts of 6×10^3 cfu/m^3 and 1.3×10^3 cfu/m^3, respectively. At all sampling points there was a noted variation in genera identified. Different genera/species were also found in CA and NCA compared with the genera found in CP (Table 4). The genera identified in the three sampling points were mostly gram positive, except for the AW season of CA where 73% of genera were gram negative (Table 5).

As a result of the 36 air samples collected, 196 mold identifications were performed. The genus *Penicillium* was the most frequent with an approximate value of 95% of total fungi count in CA. In CP, fungi were mainly divided between genera of filamentous fungi *Penicillium* and *Cladosporium* and the yeast *Rhodotorula* (90.7%). In the NCA SS, predominant genera were the same with total

TABLE 1. Mean of Total Counts (cfu/m^3) of Each Bacterial Genus Identified by Season and Sampling Point

Sampling point	Autumn/winter				Spring/summer			
	Gram	Genus	Classification	Total	Gram	Genus	Classification	Total
Critical area	(–)	*Serratia* spp.	NC	1990	(–)	*Acinetobacter* spp.	NC	1920
	(–)	*Proteus* spp.	2	1340	(–)	*Enterobacter* spp.	2	587
	(–)	*Citrobacter* spp.	NC	620	(–)	*Chryseobacterium* spp.	NC	480
	(–)	*Chromobacterium* spp.	NC	60	(–)	*Klebsiella* spp.	2	300
	(+)	*Micrococcus* spp.	NC	200	(–)	*Shewanella* spp.	NC	120
	(+)	*Bacillus* spp.	NC	182	(–)	*Stenotrophomonas* spp.	NC	20
	(+)	*Staphylococcus* spp. (negative coagulase)	NC	173	(–)	*Pseudomonas* spp.	2	20
					(+)	*Corynebacterium* spp.	2	6040
					(+)	*Micrococcus* spp.	NC	1940
					(+)	*Staphylococcus* spp. (negative coagulase)	NC	1287
					(+)	*Arthrobacter* spp.	NC	320
					(+)	*Bacillus* spp.	NC	224
	Mean of total counts of bacteria = 9.6×10^3				Mean of total counts of bacteria = 2.1×10^4			
Control point	(–)	*Acinetobacter* spp.	NC	400	(–)	*Shigella* spp.	NC	990
	(–)	*Burkholderia* spp.	NC	40	(+)	*Staphylococcus* spp. (negative coagulase)	NC	113
	(–)	*Enterobacter* spp.	2	30	(+)	*Bacillus* spp.	NC	37
	(+)	*Micrococcus* spp.	NC	143	(+)	*Corynebacterium* spp.	2	20
	(+)	*Staphylococcus* spp. (negative coagulase)	NC	51	(+)	*Micrococcus* spp.	NC	10
	(+)	*Bacillus* spp.	NC	14	(+)	*Brevibacterium* spp.	NC	10
	(+)	*Cellulomonas* spp./ *Microbacterium* spp.	NC	10	(+)	*Cellulomonas* spp./ *Microbacterium* spp.	NC	10
	(+)	*Kytococcus* spp.	NC	10	(+)	*Enterococcus* spp.	2	10
					(+)	*Gardnerella* spp.	2	10
	Mean of total counts of bacteria = 4.4×10^2				Mean of total counts of bacteria = 5.9×10^2			
Noncritical area	(–)	*Pseudomonas* spp.	2	510	(–)	*Klebsiella* spp.	2	100
	(–)	*Acinetobacter* spp.	NC	230	(–)	*Acinetobacter* spp.	NC	40
	(–)	*Serratia* spp.	NC	70	(–)	*Enterobacter* spp.	2	10
	(–)	*Klebsiella* spp.	2	50	(+)	*Staphylococcus* spp. (negative coagulase)	NC	232
	(–)	*Proteus* spp.	NC	40	(+)	*Staphylococcus* spp. (positive coagulase)	NC	120
	(–)	*Kluyvera* spp.	NC	30	(+)	*Bacillus* spp.	NC	92
	(+)	*Staphylococcus* spp. (negative coagulase)	NC	130	(+)	*Cellulomonas* spp./ *Microbacterium* spp.	NC	70
	(+)	*Micrococcus* spp.	NC	120	(+)	*Micrococcus* spp.	NC	40
	(+)	*Corynebacterium* spp.	2	85	(+)	*Brevibacterium* spp.	NC	30
	(+)	*Bacillus* spp.	NC	65	(+)	*Arthrobacter* spp.	NC	10
	(+)	*Staphylococcus* spp. (negative coagulase)	2	10				
	Mean of total counts of bacteria = 1×10^3				Mean of total counts of bacteria = 1.3×10^3			

Note. NC, nonclassified.

fungi counts of 82.3% (Table 5). *Penicillium* was not the predominant genus in the SS season.

According to the classification of biological agents (European Parliament, 2000), only three filamentous fungal genera were identified as group 2: *Microsporum* and *Trichophyton*, detected in both seasons in CP, and *Trichophyton* only in CA during SS. The predominant genus in CA (*Penicillium*) has a high presence in SS with mean counts of 1.5×10^4 cfu/m^3. In SS season a greater variability of genera of fungi was observed in

TABLE 2. Mean of Total Counts (cfu/m^3) of Each Fungal Genus Identified by Season and Sampling Point

Sampling point	Autumn/winter			Spring/summer		
	Genus	Classification	Total	Genus	Classification	Total
Critical area	*Penicillium* spp.	NC	9100	*Penicillium* spp.	NC	15093
	Cladosporium spp.	NC	1020	*Rhodotorula* spp.	NC	2440
	Aspergillus spp.	NC	70	*Ulocladium* spp.	NC	1020
				Candida spp.	NC	300
				Cladosporium spp.	NC	280
				Rhizopus spp.	NC	60
				Aspergillus spp.	NC	56
				Trichophyton spp.	2	40
	Mean of total counts of fungi = 1.1×10^4			Mean of total counts of fungi = 1.9×10^4		
Control point	*Cladosporium* spp.	NC	190	*Cladosporium* spp.	NC	1127
	Penicillium spp.	NC	93	*Rhodotorula* spp.	NC	780
	Rhodotorula spp.	NC	40	*Penicillium* spp.	NC	92
	Microsporum spp.	2	25	*Streptomyces* spp.	NC	90
	Trichophyton spp.	2	20	*Cryptococcus* spp.	NC	67
				Candida spp.	NC	55
				Trichophyton spp.	2	26
				Ulocladium spp.	NC	15
				Aspergillus spp.	NC	15
				Microsporum spp.	2	10
	Mean of total counts of fungi = 3.4×10^2			Mean of total counts of fungi = 2.4×10^3		
Noncritical area	*Penicillium* spp.	NC	853	*Rhodotorula* spp.	NC	710
	Cladosporium spp.	NC	167	*Penicillium* spp.	NC	323
	Candida spp.	NC	165	*Candida* spp.	NC	232
	Cryptococcus spp.	NC	60	*Cladosporium* spp.	NC	198
	Rhodotorula spp.	NC	20	*Ulocladium* spp.	NC	33
	Aspergillus spp.	NC	10	*Saccharomyces* spp.	NC	30
				Cryptococcus spp.	NC	25
				Aspergillus spp.	NC	15
				Trichophyton spp.	2	10
	Mean of total counts of fungi = 1.4×10^3			Mean of total counts of fungi = 1.8×10^3		

Note. NC, nonclassified.

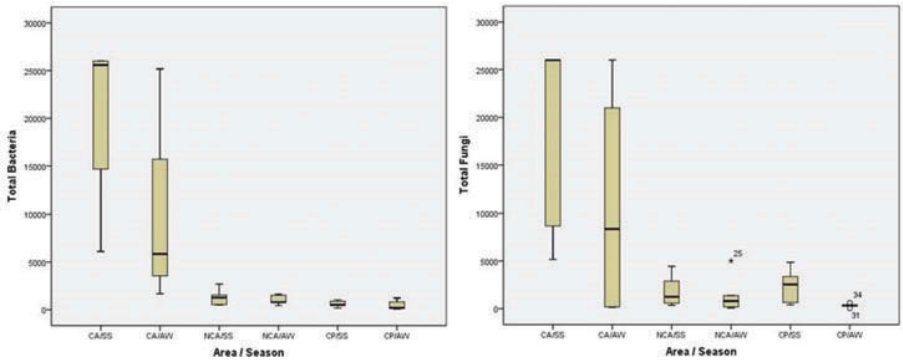

FIGURE 1. Total bacteria and fungi counts (cfu/m^3) in packaging glass sorting (mean, minimum, and maximum levels).

comparison with AW season (Table 2). Different genera/species were also found in CA and NCA compared with the genera found in CP (Table 4).

DISCUSSION

While Directive 2000/54/EC (European Parliament, 2000) focused on the principles

TABLE 3. Multiple Comparisons of Total Bacteria and Total Fungi That Are Not Significant at the .05 Level

Total bacteria			Total fungi		
Areas/season	Areas/season	Significance	(Areas/season	Areas/season	Significance
CA/SS	CA/AW	.278	CA/AW	NCA/SS	.705
				NCA/AW	.234
				CP/SS	.995
NCA/SS	NCA/AW	.509	NCA/SS	NCA/AW	.429
				CP/SS	.732
				CP/AW	.069
NCA/AW	CP/SS	.3171	NCA/AW	CP/SS	.276
CP/SS	CP/AW	.3349		CP/AW	.288

TABLE 4. Microbial Species and Genera Identified by Sampling Point and Season

Gram-negative bacteria: *Acinetobacter baumannii* (NCA/SS), *Acinetobacter lwoffi* (CA/SS + CP/AW + NCA), *Burkholderia cepacia* (CP/AW), *Chromobacterium violaceum* (CA/AW), *Chryseobacterium meningosepticum* (CA/SS), *Citrobacter freundii* (CA/AW), Enterobacter spp. (CA/SS), *Enterobacter cloacae* (CA/SS), *Enterobacter sakazakii* (CP/AW + NCA/SS), *Klebsiella* spp. (NCA/AW), *Klebsiella oxytoca* (NCA/SS), *Klebsiella pneumoniae Pneumoniae* (CA/SS + NCA/SS), *Kluyvera ascorbata* (NCA/AW), *Proteus* spp. (NCA/AW), *Proteus penniri* (CA/AW), *Proteus vulgaris* (CA/AW), *Pseudomonas aeruginosa* (CA/SS + NCA/AW), *Serratia marcescens* (CA/AW + NCA/AW), *Shewanella putrefaciens* (CA/SS), *Shigella* spp. (CP/AW), *Stenotrophomonas maltophilia* (CA/SS).

Gram-positive bacteria: *Arthrobacter* spp. (CA/SS + NCA/SS), *Bacillus* spp. (CA + CP + NCA), *Bacillus brevis* (NCA/SS), *Bacillus cereus* (CA + CP + NCA), *Bacillus licheniformis* (NCA/AW), *Bacillus subtilis* (CP/AW + NCA/AW), *Bacillus sphaericus* (CP/SS + NCA/SS), *Brevibacterium* spp. (CP/SS + NCA/SS), *Cellulomonas* spp./*Microbacterium* spp. (CP/AW + NCA/SS), *Corynebacterium* spp. (CP/SS + NCA/AW), *Corynebacterium aquaticum* (CA/SS), Corynebacterium pseudodiphtheriticum (NCA/AW), *Enterococcus avium* (CP/SS), *Gardnerella vaginalis* (CP/SS), *Micrococcus* spp. (CA + CP + NCA), *Micrococcus sedentarius* (CP/AW), *Staphylococcus aureus* (NCA/AW), *Staphylococcus* negative coagulase (CA + CP + NCA), *Staphylococcus* positive coagulase (NCA/SS).

Fungi: *Aspergillus spp.* (CA/AW), *Aspergillus niger* (CA + CP/SS + NCA), *Aspergillus flavus* (NCA/SS), *Aspergillus terreus* (CP/SS), *Candida* spp. (CA/SS + CP/SS + NCA/SS), *Candida boidinii* (NCA/AW), *Candida famata* (NCA), *Candida kefyr* (NCA/SS), *Cladosporium* spp. (CA + CP + NCA), *Cryptococcus* spp. (NCA/AW), *Cryptococcus laurentii* (CP/SS + NCA/SS), *Microsporum* spp. (CP/SS), *Microsporum equinum* (CP/AW), *Penicillium* spp. (CA + CP + NCA), *Rhizopus* spp. (CA/SS), *Rhodotorula* spp. (CA/SS + CP + NCA), *Rhodotorula glutinis* (CP/SS + NCA/AW), *Saccharomyces* spp. (NCA/SS), *Streptomyces* spp. (CP/SS), *Trichophyton* spp. (CA/SS + CP/SS + NCA/SS), *Trichophyton equinum* (CP), *Ulocladium* spp. (CA/SS + CP/SS + NCA/SS)

Note. Sampling points are given in parentheses. Quoting only the letters of the sampling point with no season means that the genus/species was found in both seasons SS/AW.

TABLE 5. Distribution (%) of Total Counts of Bacteria Detected by Gram Classification, Season, and Sampling Point

		Total % (cfu/m^3)	
Sampling point	Gram	Autumn/winter	Spring/summer
Critical area (CA)	(–)	73.0%	17.7%
	(+)	27.0%	82.3%
Control point (CP)	(–)	26.4%	31.0%
	(+)	73.6%	69.0%
Noncritical area (NCA)	(–)	21.8%	5.3%
	(+)	78.2%	94.7%

for management of biological hazards in the workplace, biological risk assessment remains one of the issues for which studies need to be carried out to enable more efficient management of all aspects of occupational risk. The importance of assessing exposure to bioaerosols is crucial, which clearly indicates the need for regular monitoring in order to identify biological agents to which workers are exposed, as reported in the "Priorities for Occupational Safety and Health Research in Europe: 2013–2020" (European Agency for Safety and Health at Work, 2013).

Exposure to bioaerosols in the waste industry was reported by several investigators (Corrao et al., 2012; Dutkiewicz, 1997; Gladding, 2002, Gladding et al. 2003a; Gladding and Coggins, 1997; Kiviranta et al., 1999; Krajewski, et al., 2002; Malta-Vacas et al., 2012; Park et al., 2011; Viegas et al.,

2014b). However, specific knowledge of exposure to biological agents according to the type of waste treated is limited. It is recognized that there are substantial differences in terms of organic breakdown depending on the type of waste handled directly influencing microbial colonization. As reported by Nersting et al. (1991), concentrations of microorganisms seemed to rise as the quality of the waste deteriorated and activity increased. Moreover, waste and the biodegradable organic material generated from it are kept in the corresponding containers for different periods before collection, providing an ideal environment for excessive microbial growth (Park et al., 2011).

Our results showed that the average count of viable bacteria and fungi in CA was markedly higher than in CP and NCA, demonstrating higher levels of biological agents at work locations involved in packaging glass sorting. The environmental microflora of CA is mainly composed of bacteria (1.6×10^4 cfu/m^3), while the average of the viable fungal counts is numerically lower (1.5×10^4 cfu/m^3). The NCA also presented high counts of viable bacteria and fungi (1.2×10^3 cfu/m^3 and 1.6×10^3 cfu/m^3), possibly due to the particular structural conditions of the company with the administrative sector being located in the same building as the manufacturing area. In addition, there was infrequent use of the ventilation and acclimatization systems in the NCA.

Natural ventilation is a feasible practice due to the storage of the hull, which takes place outside, to the south and east of the administrative sector. The high mean counts of viable bacteria and fungi at CP are possibly influenced by the hull being stored outside (5.2×10^2 cfu/m^3 and 1.3×10^3 cfu/m^3).

The sampling of bioaerosols involves removal and collection of biological particles in the atmosphere (Pillai and Ricke, 2002). There is a considerable variety of methods of sampling and analyzing bioaerosols. However, several limitations that currently exist have not been resolved, namely, lack of standardized methods and protocols that are effective in sampling and analyzing all types of bioaerosols (Buttner et al., 2002; Goyer et al., 2001). Although the short-term air sampling methodology has recognized limitations, it is a widely used method in several occupational settings (Fracchia et al., 2006; Gladding and Coggins, 1997; Kennedy et al., 2004; Mentes et al., 2009; Park et al., 2013; Solans et al., 2007; Viegas et al., 2014a, 2014b, 2014c). Thus, it becomes difficult to make a comparison of results of different studies due to differences in the type of sampling and analytical methodology.

The average concentration of viable fungi found in a study focusing on indoor airborne exposure in point-of-sale glass bottle recycling conducted in Canada from September to March was 1.1×10^3 cfu/m^3 (Kennedy et al., 2004), with the fungal levels being associated with visibly moldy bottles being broken during the recycling process. In the waste sorting CA in our study, for the same period of the year, mean fungal counts were 10-fold higher.

In other studies regarding the waste management industry, a large variation in mean values of bacterial and fungal load was noted using short-term sampling collection. Park et al. (2013) determined the airborne bacteria and fungi during waste handling, reporting 1.6×10^4 cfu/m^3 of bacteria and 1.8×10^4 cfu/m^3 of fungi. In this study, for the specific operation of waste sorting, the mean values were 3.1×10^4 cfu/m^3 for bacteria and 4.3×10^4 cfu/m^3 for fungi. In a similar investigation, Park et al. (2011) noted 2.2×10^5 cfu/m^3 and 2.4×10^4 cfu/m^3 of bacteria and fungi during sorting of household waste. Kiviranta et al. (1999) reported higher mean concentration of fungi 100-fold (1.1×10^5 cfu/m^3) in the waste processing room at the resource recovery plant handling presorted household waste than in waste collection and landfill sites. Such values are higher than the ones detected in our study (1.5×10^4cfu/m^3). High mean values in the administrative areas—offices—were also detected in the United Kingdom (10^3 cfu/m^3) (Gladding and Coggins, 1997), in the same range as those measured in the NCA in our investigation (1.6×10^3 cfu/m^3).

Seasonal variation was reported as an important parameter in the analysis of occupational bioaerosol exposure (Fracchia et al.,

2006; Nielsen et al., 1997; Oppliger et al., 2005). Several investigators took annual variation into account when designing their studies. Gladding and Coggins (1997) collected air samples during the four seasons. Prasad et al. (2004) indicated that samples taken need to be representative of the bioaerosol concentrations over area and time. Ideally, a study needs to be undertaken over a 12-mo period in order to account for seasonal and weather variations (Prasad et al., 2004). Our study was carried out in an 8-mo period covering four seasons of the year.

The composition of the waste associated with favorable climatic conditions influences proliferation of bioaerosols during the sorting process. Malmros (1997) showed that growth is enhanced during the hot season, and the amount of fluid in the containers, often called percolate, increases in the summertime. As reported by Oppliger et al. (2005) when assessing sewage workers' exposure to airborne fungi, and by Nielsen et al. (1997) on waste collectors, bioaerosol exposure to fungi occurred at higher concentrations in summer than in winter. In the present study, the mean count of viable bacteria and fungi in the SS season was higher than in AW. The CA was the sampling point with the highest values.

The mean of total counts of fungi is higher in CP compared to NCA in the SS season, probably associated with the practice of storing the hull. Hull storage outside, and its consequent mechanical movement, in conjunction with dry weather conditions, may enable the formation of organic dusts and their dispersion, contributing toward an elevation in total counts at the CP. When evaluating the degree of bacterial contamination in composting plants, Fracchia et al. (2006) found significant interaction between bacterial contamination and season. This situation was not noted in the present study, in which there are no significant seasonal differences between the CA values.

Seasonal variation and other factors were also identified as a relevant parameter in the presence of airborne gram-negative bacteria in the studies performed by Oppliger et al. (2005) and Fracchia et al. (2006). Nevertheless, a clear connection between seasonal variation and presence of gram-negative species was not detected. In our study, CA in the AW season was clearly dominated by gram-negative species, possibly endotoxin producers. At this sampling point, the genera *Proteus, Enterobacter, Klebsiella*, and *Pseudomonas* were identified.

The waste sector is known to have high levels of contamination by filamentous fungi with high growth rates that might impair not only an effective count of the number of colonies present but also identification of all the isolates. Regarding fungal flora, it was noted that the samples from outdoors (CP) exhibited *Cladosporium* as the prevailing genus, whereas in the samples from indoors, CA and NCA, the *Penicillium* genus was predominant, in agreement with Kennedy et al. (2004) and Malta-Vacas et al. (2012). In the latter study, also carried out in Portugal, *Rhizopus* spp. was also a predominant fungus in samples collected inside. In our study, this genus was identified only in CA in SS season. A similar study reported isolation of eight different filamentous fungi in air samples, with *A. niger, A fumigatus,* and *A. flavus* being the most frequent (Viegas et al., 2014b). From these species, only A. *flavus* and A. *niger* were isolated from the seven filamentous genera identified in our study.

CONCLUSIONS

This study emphasizes the importance of assessing exposure to biological agents in the waste industry, in order to identify agents to which workers are exposed and their potential effects on health. Bacteria and fungi levels varied to a large extent, depending on the environmental characteristics. The highest levels of bacterial and fungal species were found at the waste sorting CA, showing the importance of risk assessment and measurement in the early stages of designing a waste plant, in order to minimize proliferation of biological agents in the workplace. These data reinforce the need for specific training procedures to be in place

related to occupational exposure to biological agents, in conjunction with engineering, organizational, and collective preventive measures, strengthened by the implementation of protective measures.

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