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Research article

Microbial contamination in waste collection: Unveiling this Portuguese occupational exposure scenario

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ABSTRACT

Previous studies anticipated that microorganisms and their metabolites in waste will increase as a consequence of a decreased collection frequency and due to differences in what kind of waste is bagged before collection leading to an increased exposure of workers handling the waste. This study aim was to investigate the microbial contamination present in the waste collection trucks (WCT) and in the support facilities (waste collection station - WCS). It was applied a multi-approach protocol using active (air sampling by impingement and impaction) and passive (surface swabs, electrostatic dust cloths and settled dust) sampling methods. The screening of azole-resistance, the investigation of mycotoxins and the assessment of the elicited biological responses *in vitro* were also carried out aiming recognizing the possible health effects of waste collection drivers. SARS-CoV-2 detection was also performed. In WCS only air samples had contamination in all the four sampling sites (canteen, operational removal core, operational removal center, and administrative service). Among all the analyzed matrices from the WCT a higher percentage of total bacterial counts and Gram-was detected in swabs (66.93%; 99.36%). In WCS the most common species were *Penicillium* sp. (43.98%) and *Cladosporium* sp. (24.68%), while on WCT *Aspergillus* sp. (4.18%) was also one of the most found. In the azole resistance screening *Aspergillus* genera was not observed in the azole-supplemented media. SARS-CoV-2 was not detected in any of the environmental samples collected, but *Aspergillus* section *Fumigati* was detected in 5 samples. Mycotoxins were not detected in EDC from WCS, while in WCT they were detected in filters (N = 1) and in settled dust samples (N = 16). In conclusion, our study reveals that a comprehensive sampling approach using active and passive sampling (e.g. settled dust sampling for a representative mycotoxin evaluation) and combined analytic methods (i.e., culture-based and molecular) is an important asset in microbial exposure assessments. Concerning the waste collection exposure scenario, the results of this study unveiled a complex exposure, particularly to fungi and their metabolites. *Aspergillus* section *Fumigati* highlight the significance of targeting this section in the waste management industry as an indicator of occupational health risk.

1. Introduction

Waste management industries are crucial to accomplish the European Union milestones and Sustainable Development Goals (SDGs) suggested by World Health Organization. Additionally, as in all European countries, Portuguese employers are obliged by regulation to assess and prevent exposure to occupational risks (Directive 89/391/EEC., 1989) and specifically to biologic agents the Directive 2000/54/EC of the European Parliament and the Council of September 18, 2000 sets the rules regarding risk assessment if exposure to biological agents cannot be avoided (Directive 2000/54/EC, 2000).

However, while improved waste management is being foreseen as a

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crucial contribute to reduce health outcomes and environmental negative impacts, the microorganisms occupational exposure in waste management industries is being neglected leading to negative impact on workers' health. In contrast to the effects caused by chemical and physical agents, those of biological origin are less recognized and not so well described (Domingo and Nadal, 2009). However, in humans they may cause a wide range of health outcomes, since they can act as infectious, allergenic, toxic, and carcinogenic (Schlosser and Huyard, 2008; Domingo and Nadal, 2009). Thus, handling of solid waste in traditional waste collection promotes microbial exposure, which is considered an occupational health threat (Binion and Gutberlet, 2012) due the association with several health effects, mainly respiratory ones (Poole and Basu, 2017; Thakur et al., 2018).

Previous studies anticipated that microorganisms and their metabolites (e.g mycotoxins and endotoxins) in waste will increase as a consequence of a decreased collection frequency (Gladding and Gwyther, 2017; Madsen et al., 2021) and due to differences in what kind of waste is bagged before collection (Madsen et al., 2019, 2021) leading to an increased exposure of workers handling the waste. Additionally, the presence of SARS-CoV-2 in waste management industry, due to the still ongoing pandemic crises, is a public and occupational health concern in all the waste streams and sectors of this industry, since the pandemic virus can remain viable and with infectious potential in aerosols for hours and on surfaces up to days (Van Doremalen et al., 2020). Thus, more studies are needed to characterize the virus' exposure through all the waste management chain (Vaverková et al., 2021).

Some workers handling waste, such as garbage collectors and truck drivers transporting waste produced in households, spend their work shift partly in a truck cabin and partly in a work place where organic material is handled. In taxis and cars, the sampling approach has previously been focused on ventilation filters as sources of exposure (Li et al., 2013; Viegas et al., 2018c), as well as in fork lifters operating in waste sorting units (Viegas et al., 2017, 2020h). Although it was possible to characterize de microbial contamination on the filters from the fork lifters handling the waste pile, using culture based-methods and high-throughput DNA sequencing (Viegas et al., 2017, 2020h), mycotoxins were not detected as it was expected due to biomonitoring data from the workers driving the same fork lifters (Viegas et al., 2014). In addition, *in vitro* testing of environmental samples using relevant cell cultures might provide critical information on biological responses due to co-exposure to multiple stressors present in a specific occupational environment (Segura et al., 2009; Viegas et al., 2017). It was reported that electrostatic dust collectors (EDC) collected in Danish dwellings with higher fungal contamination elicited higher total proinflammatory responses (Madsen et al., 2012). Cytotoxicity of the settled dust collected in different workstations from a waste sorting plant (Szulc et al., 2022), as well as filtering respiratory protection devices used by waste sorting workers (Viegas et al., 2020h, 2021f), against a human epithelial lung cell lines was determined in previous studies.

This study aim was to investigate the microbial contamination present in the waste collection trucks (WCT) and in the support facilities (waste collection station - WCS). The study was performed in Lisbon area applying a multi-approach protocol using active (air sampling by impingement and impaction) and passive (surface swabs, electrostatic dust cloths and settled dust) sampling methods. The screening of azole-resistance, the investigation of mycotoxins in environmental samples and the assessment of the elicited biological responses *in vitro* were also carried out aiming recognizing the possible health effects of waste collection drivers. Driven by the pandemic occurring during the sampling campaign, SARS-CoV-2 detection was also performed.

2. Materials and methods

2.1. Waste collection station and trucks assessed

This study sampling campaign was conducted between April and May 2021 in one waste collection station (WCS) located in Lisbon and in 32 waste collection trucks (WCT) from a total of 231 (13.9%). It is part of an enlarged exploratory study with financial support aiming to characterize the occupational exposure of workers to microorganisms and their metabolites enrolled in waste management activities.

Waste collection and street cleaning in the city of Lisbon are the main tasks performed by 894 workers. Among them, 267 are drivers of the waste collection trucks. All workers operate 7 days/week in a daily regimen of two 8-h shifts. On average, 310 tons of garbage are collected annually in the city of Lisbon and approximately 75% is undifferentiated garbage.

2.2. Sampling approach and characterization through culture dependent-methods

A multi-sampling approach protocol was done using active and passive sampling methods and during normal working days (Fig. 1). The impinger Coriolis μ air sampler (Bertin Technologies, Montigny-le-Bretonneux, France) was used only for SARS-CoV-2 detection in WCT. The Millipore air sampler tester (Millipore, Billerica, MA, USA) was used only in WCS with four different culture media selective of fungi and bacteria, namely: Malt extract agar (MEA) supplemented with chloramphenicol (0.05%) and dichloran-glycerol agar (DG18) were used for fungi (MEA and DG18, 27 °C, 5–7 days); tryptic soy agar (TSA) supplemented with nystatin (0.2%), and Violet Red bile agar (VRBA) for mesophilic (TSA, 30 °C, 7 days) and Gram-negative (VRBA, 35 °C, 7 days) bacteria selectivity.

Samples of WCS indoor air were collected in specific areas, such as: canteen, operational removal core dedicated to workers' locker rooms and administrative offices, operational removal center where the trucks are parked, by collecting 250 L at a flow rate of 140 L/min with the Millipore air tester, according to manufacturer's instructions (Viegas et al., 2021a) (Fig. 1).

Different passive sampling methods were employed in the sampling campaign dedicated to WCT (17 EDC, 82 surfaces swabs, 32 settled dust filters and 1 settled dust) and WCS (18 surface swabs and 18 EDC) (Fig. 1).

Concerning settled dust collection, a vacuum cleaner (HOOVER Brave BV71_BV10 A2, USA) with 1 × 4 collector filter attached (used for further analyses) and a composite sample of the settled dust from the driver's cabin from all the WCT was obtained (Viegas et al., 2021a,e,f) (Fig. 1).

The surfaces from the waste collection station (the same sampling sites used for air sampling) and from each WCT (one swab between the seats) were swabbed, using sterile cotton swabs moistened in sterilized water, using a 10 × 10cm square stencil, disinfected between samplings with a 70% alcohol solution (ISO, 18593:, 2004) to allow quantification. The EDC were placed in each sampling site of the WCS and in the drivers' cabin from WCT, next do the driver, for 30 days. All samples obtained (EDC, filters, settled dust) were preserved in sterilized bags or in transport tubes (swabs) and transported under refrigeration (0-4 °C) to the laboratory for further analyses (Figs. 1 and 2) (Viegas et al., 2021e) (Figs. 1 and 2).

Swabs were washed with 1 mL of 0.1% Tween 80 saline (0.9% NaCl) for 30 min on the orbital shaker (250 rpm). The same procedure was applied on a piece (2 cm^2) of each filter used from vacuuming. A composite sample with the settled dust obtained from each WCT was washed in a ratio of 1 g per 9.1 mL of 0.1% Tween 80 saline (0.9% NaCl) for 30 min at 250 rpm (Viegas et al., 2021f). EDC were weighted and processed with 20 mL of the same washing solution.

After incubation of MEA, DG18 at 27 °C for 5–7 days for fungi, and TSA and VRBA at 30 °C and 35 °C for 7 days for mesophilic bacteria and coliforms (Gram negative bacteria), respectively, microbial contamination densities (colony-forming units, CFU. g^{-1}, CFU. m^{-2}, CFU. m^{-2}. day^{-1}) were calculated as previously reported (Viegas et al., 2021d). Fungal species were identified microscopically using tease mount or

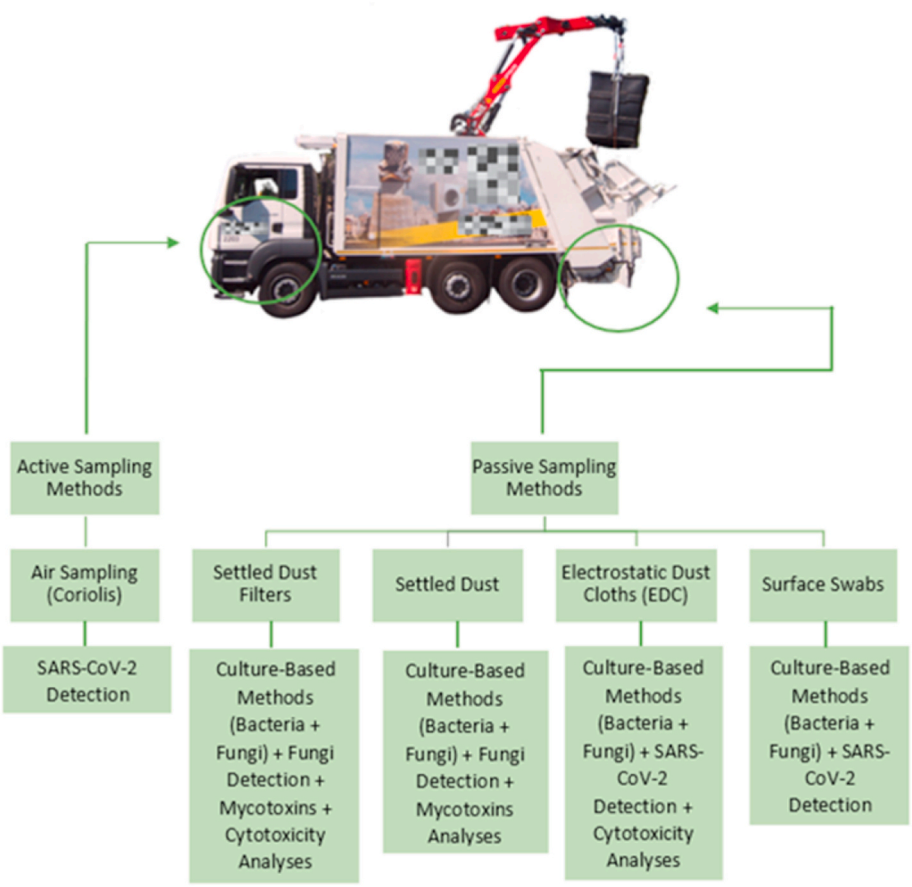

Fig. 1. WCT sampling and analysis performed.

Scotch tape mount and lactophenol cotton blue mount procedures. Morphological identification was achieved through macro and microscopic characteristics as noted by De Hoog et al. (2016). Negative controls were employed to ensure the inexistence of background contamination, namely: culture media (all samples) and extracts of control samples (swabs, EDC, vacuuming filters) without prior use were submitted to the same assays.

2.3. Azole resistance screening

The susceptibility to azoles of fungi obtained from 82 swabs and 35 EDC from WCS and WCT and 32 settled dust filters from WCT (N = 149) was tested using azole screening media, according to the EUCAST breakpoints (Arendrup et al., 2013; European Committee on Antimicrobial Susceptibility Testing, 2020). Sabouraud dextrose agar (SDA) (Frilabo, Maia, Portugal) supplemented with 4 μg/mL itraconazole (ITZ), 2 μg/mL voriconazole (VCZ), and 0.5 μg/mL posaconazole (PSZ) was used to inoculate the samples (150 μL) and incubated at 27 °C for four days. As control, an SDA plate without azoles was used. The reference strains used were kindly provided by National Health Institute Doutor Ricardo Jorge, IP, and consisted of: *A. fumigatus* ATCC 204305 (negative control); and pan-azole-resistant *A. fumigatus* (positive control). Fungal assessment was performed by colony observation and counting and microscopical identification, as previously described (Viegas et al., 2020a). Fungi not able to grow in the azole-supplemented plates were considered susceptible to the respective azoles, according to EUCAST guidelines (European Committee on Antimicrobial Susceptibility Testing, 2020).

2.4. Sampling and molecular detection of SARS-CoV-2 and targeted Aspergillus sections

Regarding SARS-CoV-2, composite surface samples were collected by swabbing specific areas from each sampling site in the WCS and from each WCT (steering wheel, control buttons, handles, knobs) (Fig. 3), using sterile cotton swabs moistened in Buffer NVL (SARS-COV-2 assessment) and following the same procedures applied on swabs performed to assess fungi and bacteria.

Air samples of 600L were collected using the impinger Coriolis μ air sampler (Bertin Technologies, Montigny-le-Bretonneux, France) with a flow rate of 300 L/min collected into a conical vial containing 5 mL Buffer NVL (NZY Viral RNA Isolation kit (MB40701) component).

RNA was extracted from the isolated sample (5 mL in air samples and 1.5 mL in surface samples) with the NZY Viral RNA Isolation kit, from Nzytech, according to manufacturer's instructions. One step-RT qPCR was performed using NZYSpeedy One-step RT-qPCR probe Master Mix with primers and probes published by CDC (available on https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html), which detect two viral gene regions (N1 and N2). qPCR was performed on BioRad CFX96 PCR machine. In each analysis a positive (a SARS-CoV-2 positive sample) and a negative (water) sample was included. Moreover, in order to detect possible PCR inhibitors an internal control was added to each PCR.

Samples extracts (8.8 mL) from passive sampling (excluding surface swabs) were used for molecular detection of *Aspergillus* sections (Viegas et al., 2021c). Fungal DNA was extracted using the ZR Fungal/Bacterial DNA MiniPrep Kit (Zymo Research, Irvine, USA) and molecular identification was performed by Real Time PCR (qPCR) using the CFX-Connect PCR System (Bio-Rad). Reactions included 1 × iQ Supermix (Bio-Rad, Portugal), 0.5 μM of each primer, and 0.375 μM of TaqMan probe in a

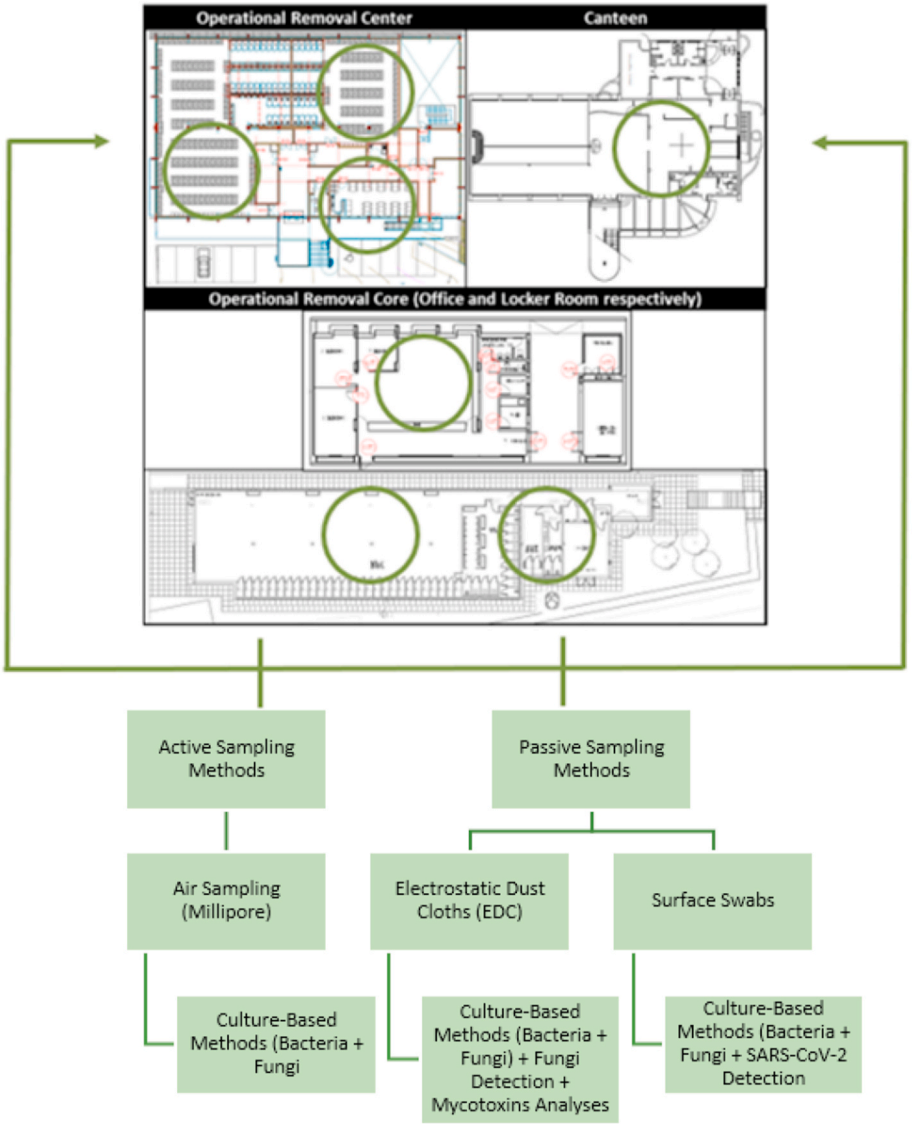

Fig. 2. WCS sampling and analysis performed.

total volume of 20 μl. Amplification followed a three-step PCR: 40 cycles with denaturation at 95 °C for 30 s, annealing at 52 °C for 30 s, and extension at 72 °C for 30 s.

A non-template control and a positive control consisting of DNA obtained from a reference that belonged to the culture collection of the Reference Unit for Parasitic and Fungal Infections, Department of Infectious Diseases of the National Institute of Health, from Dr. Ricardo Jorge. These strains have been sequenced for ITS, B-tubulin, and Calmodulin.

2.5. Mycotoxins analysis

A total of 18 samples from WCS (18 EDC: 5 from administrative offices, 3 from canteen, 4 from operational removal core and 4 from operational removal center) and 64 samples from WCT (32 settled dust filters and 32 settled dust) were screened for mycotoxins presence. In all samples, 38 mycotoxins were analyzed by HPL-MS ((HPLC) Nexera (Shimadzu, Tokyo, Japan) with a mass spectrometry detector API 4000 (Sciex, Foster City, CA, USA) following the same laboratories procedures described in previous papers (Viegas et al., 2021d). The mycotoxin concentration was calculated using external calibration. The Limits of Detection (LOD) obtained for each mycotoxin with the analytical method used are presented in Table S1 - Supplemental material.

2.6. Cytotoxicity analyses

Cytotoxicity evaluation was carried out using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay. This assay determines the metabolic activity of cells by means of enzymatic reduction of the tetrazolium dye MTT at 510 nm, relating to the number of viable cells (Hanelt et al., 1994). Human lung epithelial (A549) and swine kidney (SK) cells were maintained in Eagle's Minimum Essential Medium (MEM) supplemented with 10,000 units penicillin and 10 mg/mL streptomycin in 0.9% NaCl and fetal bovine serum (Sigma-Aldrich, USA). Cells were detached with 0.25% (w/v) Trypsin 0.53 mM EDTA, suspended in the culture medium and counted (Scepter™ 2.0 Cell Counter, Merck). Afterwards, 100 μL cell suspension was transferred to a 96-well plate (densities of 2,5 x 10^5 cells/ml) and exposed to up to five dilutions of EDC samples for 48 h at 5% CO2, 37 °C, and humid atmosphere. Regarding filter samples, 1 mL of the sample was used for the MTT test. Then, MTT stock solution (20 μL) was added to each well, and plates were incubated for another 4 h. After removing supernatant with a multichannel micropipette, 100 μL DMSO was added to each well, and absorbance was measured spectrophotometrically with

Fig. 3. WTC sampled areas.

an ELISA-Reader (ELISA LEDETECT 96, biomed Dr. Wieser GmbH; MikroWin, 2013SC software). The threshold toxicity level was considered the lowest concentration dropping absorption to <50% of cell metabolic activity (IC50).

2.7. Statistical analysis

Data were analyzed using SPSS statistical software, V. 26.0 for Windows. The results were considered significant at the 5% significance level. To test the normality of the data, the Shapiro-Wilk test was used. To characterize the data, frequency analysis (n, %) and graphical representations appropriate to the nature of the data were used. To study the relationship between bacterial contamination, fungal contamination and azole resistance screening, the Spearman correlation coefficient was used, since the assumption of normality was not verified. To compare bacterial contamination, fungal contamination and azole resistance screening between the sampling methods, the Kruskal-Wallis test was used, since the assumption of normality was not verified.

3. Results

3.1. Viable bacterial contamination on WCS and WCT

In WCS only Millipore samples had contamination in all the four sampling sites (canteen, operational removal core, operational removal center, and administrative service). The prevalence on TSA was higher in the administrative service (38.31%), followed by the operational removal core (29.21%), operational removal center (19.54%) and canteen (12.94%). In VRBA media, the prevalence was the same (33.33%) in all sampling sites, except in the Canteen, where there were no results for bacterial contamination (Fig. 4).

Among all the analyzed matrices from the WCT and considering results obtained in TSA, a higher percentage of total bacterial counts was detected in swabs (66.93%), followed by EDC (22.86%) and settled dust (10.21%). In VRBA, the higher prevalence of Gram-negative bacteria contamination was observed in swabs (99.36%) (Fig. S1 – Supplementary material).

3.2. Viable fungal contamination on WCS and WCT

Fungal contamination in Millipore samples (the only samples with viable contamination) from WCS ranged from $1.43x10^4$ to 2.12×10^3 CFU m^{-3} in MEA, being the highest value for the administrative service and the lowest for the canteen. Concerning DG18 the fungal counts ranged between $2.05x10^3$ and 1.04×10^2 CFU m^{-3}. The highest value belongs to the operational removal center (2.05×10^3 CFU m^{-3}) and the lowest to canteen (1.04×10^2 CFU m^{-3}) (Fig. S2 - Supplementary material).

MEA presented higher diversity than DG18 (MEA - 27 species; DG18 - 18 species). In MEA 8 species were detected in the operational removal core and operational removal center, 6 in the administrative services and 5 in the canteen. In DG18 7 species were identified in the operational removal center, 4 in the canteen and administrative services and 3 in the operational removal core (Fig. S3 - Supplementary material).

The most common species in both culture media were *Penicillium* sp. (MEA 33.45%; DG18 36.82%) and *Cladosporium* sp. (MEA 14.62%;

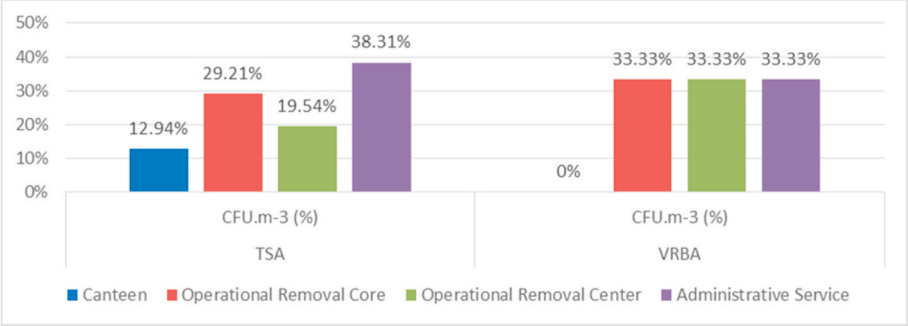

Fig. 4. Bacteria distribution on TSA and VRBA in Millipore samples from different sites (canteen, operational removal core, operational removal center and administrative service) from the assessed WCS.

DG18 58.07%).

In the canteen the most common in MEA were *Chrysosporium* sp. (94.34%) and *Cladosporium* sp. (3.40%), while in DG18, the most prevalent were *Penicillium* sp. (46.15%) and *Cladosporium* sp. (38.46%). Regarding the operational removal core, the most common on MEA and DG18 were *Cladosporium* sp. (MEA 51.67%; DG18 39.39%) and *Penicillium* sp. (MEA 47.59%; DG18 51.52%). In the operational removal center the most prevalent in MEA were *Penicillium* sp. (59.46%) and *Aureobasidium* sp. (19.55%), while in DB18 the most prevalent were *Cladosporium* sp. (88.67%) and *Penicillium* sp. (6.84%) (Table 1). In the Administrative Service, the most prevalent in MEA was *Aureobasidium* sp. (32.61%), *Cladosporium* sp. (17.12%) and *Penicillium* sp. (17.54%), while in DG18 the most prevalent was *Penicillium* sp. (91.83%).

On MEA the *Aspergillus* sections observed were *Flavi* in the operational removal core (14.29%) and operational removal center (28.57%), *Nidulantes* and *Nigri* (28.57%) in the operational removal center (Fig. 5).

Regarding DG18, the *Aspergillus* sections found were *Aspergilli* (11.11%) in the canteen, *Flavi* in the operational removal core (11.11%), *Nidulantes* in operational removal center (22.22%) and *Circumdati* in the administrative service (55.56%) (Fig. 5).

Fungal contamination on WCT ranged from $2.65x10^6$ (swab samples) to 1.33×10^4 CFU m^{-2} (EDC) in MEA. Concerning DG18, the fungal counts ranged between $2.65x10^6$ (settled dust samples) and 1.11×10^4 CFU m^{-2} (EDC) (Fig. S4 - Supplementary material).

MEA presented higher diversity than DG18 (MEA - 29 fungi/species/sections; DG18 - 23 fungi/species/sections). In MEA 11 were detected on settled dust and 9 on swabs and EDC. In DG18 10 were identified on swabs, 7 on settled dust and 6 on EDC.

The most common in both culture media were *Cladosporium* sp. (32.94%) (MEA 42.28%; DG18 23.75%), *Penicillium* sp. (28.90%) (MEA 32.17%; DG18 26.0%) and *Aspergillus* sp. (4.18%) (MEA 0.85%; DG18 7.13%). *C. sitophila* was only prevalent in DG18 (40.35%).

In swab samples the most common in MEA were *Cladosporium* sp. (43.40%) and *Penicillium* sp. (41.13%), while in DG18, the most prevalent were *Penicillium* sp. (45.35%), *Cladosporium* sp. (33.14%), *Aspergillus* sp. (13.37%) and *Fusarium* sp. (6.98%). Regarding settled dust samples, the most common species on MEA and DG18 were also *Cladosporium* sp. (MEA 42.86%; DG18 17.44%) and *Penicillium* sp. (MEA 12.93%; DG18 13.32%). The EDC samples showed higher prevalence of *Cladosporium* sp. (MEA 60%; DG18 54.29%) and *Penicillium* sp. (MEA 14.40%; DG18 29.52%) (Table 2).

On MEA the *Aspergillus* sections found were *Fumigati* (51.3%) and *Nigri* (48.7%). These two sections were observed in surface swab samples (*Fumigati* and *Nigri* - 50%), settled dust samples (*Fumigati* - 56%; *Nigri* - 44%) and EDC (Nigri – 100%) (Fig. 5).

Regarding DG18, the *Aspergillus* sections found were *Fumigati* (3.29%), *Nigri* (9.87%), *Aspergilli* (36.7%), *Candidi* (13.4%), *Circumdati* (25.12%) and *Flavi* (11.85%). In surface swabs all the sections mentioned above were detected (*Fumigati* 4.35%; *Nigri* 13.04%; *Aspergilli* 47.83%; *Candidi* (17.39%) *Circumdati* 8.70%; *Flavi* 8.70%). On settled dust, the *Aspergillus* sections found were *Flavi* (19.63%), *Aspergilli* (1.84%) and *Circumdati* (78.53%). In EDC, the only *Aspergillus* section found was *Circumdati* (100%) (Fig. 6).

Table 1
Fungal distribution on MEA and DG18 in the different sampling sites from WCS.

SAMPLING SITE	MEA			DG18		
	Fungi	CFU.m^{-3}	%	Fungi	CFU.m^{-3}	%
Canteen	*Cladosporium* sp.	7,20E+01	3.40%	*Alternaria* sp.	1,20E+01	11.54%
	Penicillium sp.	4,00E+01	1.89%	*A.* section *Aspergilli*	4,00E+00	3.85%
	Aureobasidium sp.	4,00E+00	0.19%	*Cladosporium* sp.	4,00E+01	38.46%
	Chrysosporium sp.	2,00E+03	94.34%	*Penicillium* sp.	4,80E+01	46.15%
	Phoma sp.	4,00E+00	0.19%			
Operational Removal Core	*Cladosporium* sp.	2,23E+03	51.67%	*Cladosporium* sp.	1,04E+02	39.39%
	Penicillium sp.	2,06E+03	47.59%	*Penicillium* sp.	1,36E+02	51.52%
	A. section *Flavi*	4,00E+00	0.09%	*Chrysosporium* sp.	2,40E+01	9.09%
	Other species	2,80E+01	0.65%			
Operational Removal Center	*Cladosporium* sp.	1,32E+02	1.28%	*Cladosporium* sp.	1,82E+03	88.67%
	Penicillium sp.	6,13E+03	59.46%	*Penicillium* sp.	1,40E+02	6.84%
	Aureobasidium sp.	2,02E+03	19.55%	*A.* section *Flavi*	4,00E+00	0.20%
	A. section *Flavi*	8,00E+00	0.08%	*A.* section *Nidulantes*	8,00E+00	0.39%
	A. section *Nidulantes*	8,00E+00	0.08%	*A.* section *Aspergilli*	4,40E+01	2.15%
	A. section *Nigri*	8,00E+00	0.08%	Other species	3,60E+01	1.76%
	Other species	2,01E+03	19.47%			
Administrative Service	*Cladosporium* sp.	2,10E+03	17.12%	*Cladosporium* sp.	4,00E+01	3.89%
	Penicillium sp.	2,15E+03	17.54%	*Penicillium* sp.	9,44E+02	91.83%
	Aureobasidium sp.	4,00E+03	32.61%	*A.* section *Circumdati*	2,00E+01	1.95%
	Other species	4,02E+03	32.74%	*Chrysosporium* sp.	2,40E+01	2.33%

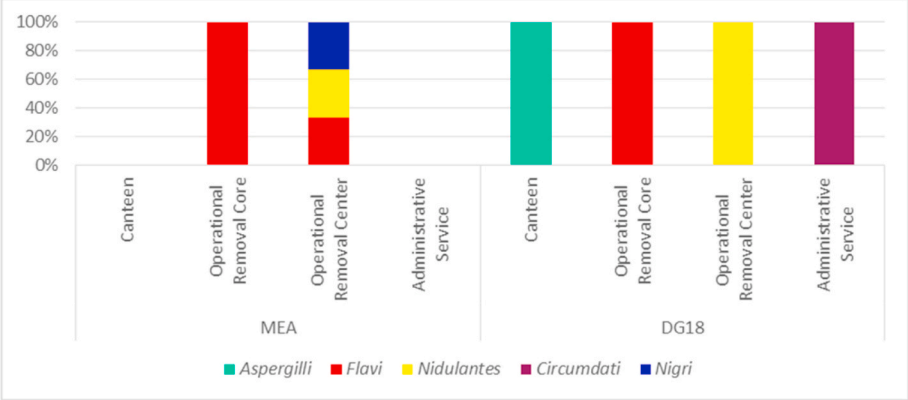

Fig. 5. Aspergillus sections on WCS air in MEA and DG18.

Table 2
Fungal distribution on MEA and DG18 in the different environmental samples from WCT.

SAMPLE	MEA			DG18		
	Fungi	CFU.m^{-2}/g/m^{-2}.day^{-1}	%	Fungi	CFU.m^{-2}/g/m^{-2}.day^{-1}	%
SWABS	*Cladosporium* sp.	1.2E+06	43.40%	*Cladosporium* sp.	5.7E+05	33.14%
	Penicillium sp.	1.1E+06	41.13%	*Penicillium* sp.	7.8E+05	45.35%
	Aspergillus sp.	2.0E+04	0.75%	*Aspergillus* sp.	2.3E+05	13.37%
	C. sitophila	1.3E+05	4.91%	*C. sitophila*	2.E+04	1.16%
	Other species	2.6E+05	9.81%	*Fusarium* sp.	1.2E+05	6.98%
SETTLED DUST	*Cladosporium* sp.	5.2E+05	4.86%	*Cladosporium* sp.	4.6E+05	17.44%
	Penicillium sp.	1.6E+05	12.93%	*Penicillium* sp.	3.5E+05	13.32%
	Aspergillus sp.	1.3E+04	1.02%	*Aspergillus* sp.	8.2E+04	3.08%
	C. sitophila	1.5E+04	1.23%	*C. sitophila*	1.8E+06	66.16%
	Other species	5.1E+05	41.96%			
EDC	*Cladosporium* sp.	8.0E+03	60.00%	*Cladosporium* sp.	6.1E+03	54.29%
	Penicillium sp.	1.9E+03	14.40%	*Penicillium* sp.	3.3E+03	29.52%
	Aspergillus sp.	6.4E+02	4.80%	*Aspergillus* sp.	3.2E+02	2.86%
	C. sitophila	1.1E+03	8.00%	*C. sitophila*	6.4E+02	5.71%
	Other species	1.7E+03	12.80%	Other species	8.5E+02	7.62%

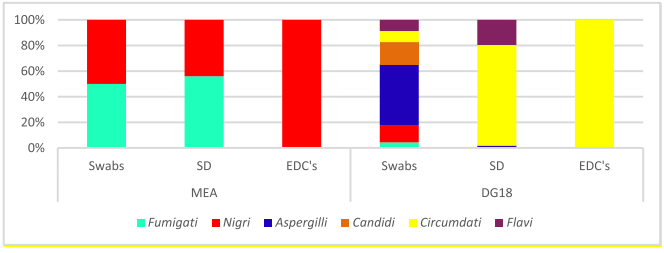

Fig. 6. *Aspergillus* sections in both culture media (MEA and DG18) from WCT.

3.3. Azole resistance profile

Considering azole-resistant fungal species distribution in the waste trucks, regardless sampling strategy, the most frequent fungal genera/species were *Cladosporium* sp. in SDA (44.0%) and in VCZ (53.4%), and *C. sitophila* in ITZ (53.1%) and PSZ (57.6%). Regarding the *Aspergillus* genus, it was only observed in SDA (0.7% in all sampling), including the following sections: *Nigri* (in swabs, settled dust filters, EDC); *Circumdati* and *Flavi* (in settled dust filters); and *Fumigati* (in settled dust filters and EDC).

As for fungal species' distribution in azole-supplemented media by sampling type, it is detailed in Table 3.

3.4. Detection of SARS-CoV-2 and the targeted fungal sections

SARS-CoV-2 was not detected in any of the environmental samples collected. Regarding the four *Aspergillus* sections investigated, only one was detected. *Aspergillus* section *Fumigati*, was detected in EDC samples (3 out of 16; 18.8%) and in settled dust filter samples (2 out of 32; 6.3%) (Table S2 - Supplementary material).

3.5. Mycotoxins contamination in WCS and WCT

Mycotoxins were not detected in EDC from WCS, while in both types of samples (filters and settled dust samples) from WCT they were detected. However, in the settled dust filters from WCT only one mycotoxin was detected in one sample. The mycotoxin detected was fumonisin B1 with a value below the LOQ (12 ng/g). Concerning WCT settled dust samples, 16 samples (53.1%) presented contamination. The most detected mycotoxin (in 16 samples, 50%) was mycophenolic acid with values ranging from below the LOQ (20 ng/g) to 170.1 ng/g. Sterigmatocystin, deoxynivalenol and zearalenon were detected in only one sample each with values of below the LoQ for sterigmatocystin (6 ng/g) and deoxynivalenol (35 ng/g) and 6.1 ng/g for zearalenon. In one sample was possible to observe contamination by two mycotoxins, namely mycophenolic acid and deoxynivalenol.

3.6. Cytotoxicity evaluation

The IC50 of EDC samples (n = 16) ranged from 5 mm^2/mL to 10 mm^2/mL in A549 cells, and 5 mm^2/mL in SK cells (Table 4). For filter samples (n = 32), no IC50 was calculated because the sample equivalent was unknown. Nevertheless, filter cytotoxicity was absent in most tested samples, namely, in 53% samples in A549, and in 91% filters in SK cells (data not shown).

Table 3
Fungal species' distribution in azole-supplemented media by sampling type.

Sample	Fungi	SDA	ITZ	VCZ	PSZ
SWABS CFU/m^2 (%)	*C.sitophila*	4.7E+05 (19.0%)	3.5E+05 (72.9%)	6.0E+04 (15.4%)	3.1E+05 (79.5%)
	Cladosporium sp.	1.0E+06 (40.5%)	1.1E+05 (22.9%)	1.6E+05 (41.0%)	8.0E+04 (20.5%)
	Penicillium sp.	7.9E+05 (32.0%)	1.0E+04 (2.1%)	1.5E+05 (38.5%)	0.0 (0.0%)
	Aspergillus sp.	1.0E+04 (0.4%)	0.0 (0.0%)	0.0 (0.0%)	0.0 (0.0%)
	Other species	2.0E+05 (8.1%)	1.0E+04 (2.1%)	2.0E+04 (5.1%)	0.0 (0.0%)
SETTLED DUST FILTERS CFU/g (%)	*C.sitophila*	2.7E+04 (5.0%)	1.5E+03 (0.8%)	0.0 (0.0%)	6.5E+03 (4.3%)
	Cladosporium sp.	3.2E+05 (60.4%)	1.7E+05 (91.6%)	1.3E+05 (84.6%)	1.4E+05 (92.1%)
	Penicillium sp.	1.6E+05 (29.4%)	1.0E+04 (5.4%)	1.9E+04 (11.9%)	1.0E+03 (0.7%)
	Aspergillus sp.	1.1E+04 (2.0%)	0.0 (0.0%)	0.0 (0.0%)	0.0 (0.0%)
	Other species	1.7E+04 (3.2%)	4.0E+03 (2.2%)	5.5E+03 (3.5%)	4.5E+03 (3.0%)
EDC CFU/ m^2/day (%)	*C.sitophila*	2.3E+03 (19.8%)	2.1E+03 (71.4%)	8.5E+02 (27.6%)	1.9E+03 (90.0%)
	Cladosporium sp.	3.4E+03 (28.8%)	6.4E+02 (21.4%)	1.7E+03 (55.2%)	2.1E+02 (10.0%)
	Penicillium sp.	5.5E+03 (46.8%)	1.1E+02 (3.6%)	3.2E+02 (10.3%)	0.0 (0.0%)
	Aspergillus sp.	4.2E+02 (3.6%)	0.0 (0.0%)	0.0 (0.0%)	0.0 (0.0%)
	Other species	1.1E+02 (0.9%)	1.1E+02 (3.6%)	2.1E+02 (6.9%)	0.0 (0.0%)

EDC, electrostatic dust collector; SDA, Sabouraud dextrose agar (SDA) media; ITZ, 4 μg/mL itraconazole; VCZ, 2 μg/mL voriconazole (VCZ); PSZ, 0.5 μg/mL Posaconazole.

Table 4
Distribution of threshold toxicity level (IC50) in EDC samples.

Sampling	IC50	A549	SK
		N	N
EDC	10 mm^2/mL	4	0
	5 mm^2/mL	3	3
	No cytotoxicity	10	14

3.7. Correlation and comparison analyses

In swabs for bacterial counts, only a significant positive and weak correlation was detected between TSA and VRBA ($r_S = 0.283$, $p = 0.023$), revealing that higher TSA counts are related to higher VRBA counts (Table 5). As for fungal contamination, the following significant correlations were detected, in a positive sense and of intensity that varied between weak and strong: i) MEA with DG18 ($r_S = 0.733$, p=<0.001), indicating that higher contents in MEA are related with higher counts in DG18; ii) MEA with SDA ($r_S = 0.392$, $p = 0.001$), showing that higher counts in MEA are related to higher counts in SDA iii) DG18 with fungal growth in SDA ($r_S = 0.485$, $p < 0.001$), in ITZ ($r_S = 0.274$, $p = 0.028$) and in VCZ ($r_S = 0.254$, $p = 0.043$), revealing that larger counts in DG18 media are related to higher counts in SDA, ITZ and VCZ (Table 5). With regard to azole resistance screening, significant positive correlations of weak intensity were detected between SDA and PSZ ($r_S = 0.265$, $p = 0.035$) and ITZ and VCZ media ($r_S = 0.393$, $p = 0.001$), which reveals that higher fungal counts in one media are related to higher counts in the other (Table 5).

In settled dust and regarding bacterial contamination, the following significant correlations were detected, in a positive sense and of intensity that varied between weak and moderate: i) between TSA and fungal counts in MEA ($r_S = 0.352$, $p = 0.038$); ii) TSA with fungal counts in DG18 ($r_S = 0.506$, $p = 0.002$); iii) TSA with fungal resistance in SDA ($r_S = 0.507$, $p = 0.002$); iv) TSA with fungal resistance in ITZ ($r_S = 0.411$, $p = 0.014$). These results revealed that higher bacterial counts in TSA are related to higher fungal counts in both MEA and DG18 and with higher fungal resistance counts in SDA and ITZ (Table 5).

Concerning fungal contamination, the following significant correlations were detected, in a positive sense and of intensity that varied between weak and strong: i) MEA media with DG18 ($r_S = 0.686$, $p < 0.0001$) and with SDA ($r_S = 0.704$, $p < 0.0001$), ITZ ($r_S = 0.657$, $p < 0.0001$), VCZ ($r_S = 0.447$, $p = 0.007$) and PSZ ($r_S = 0.348$, $p = 0.041$), revealing that higher fungal counts in MEA are related with higher fungal counts in DG18, SDA, ITZ, VCZ and PSZ; ii) DG18 with SDA ($r_S = 0.535$, $p = 0.001$), ITZ ($r_S = 0.572$, $p < 0.0001$), VCZ ($r_S = 0.376$, $p = 0.007$) and PSZ ($r_S = 0.398$, $p = 0.018$), revealing that higher fungal counts in DG18 are related with higher fungal counts in SDA, ITZ, VCZ and PSZ (Table 5). As for fungal counts in azole resistance screening, the following significant correlations were detected, in a positive sense and moderate intensity: i) SDA with ITZ ($r_S = 0.576$, p=<0.0001) and with VCZ ($r_S = 0.586$, $p < 0.0001$), indicating that higher fungal counts in SDA are related with higher counts in ITZ and VCZ; ii) ITZ with VCZ ($r_S = 0.472$, $p = 0.004$) and with PSZ ($r_S = 0.501$, $p = 0.002$), showing that higher fungal counts in ITZ are related with higher counts in VCZ and PSZ; iii) VCZ media with PSZ ($r_S = 0.452$, $p = 0.006$), revealing that higher fungal counts in VCZ media are related with higher counts in PSZ (Table 5).

In EDC and regarding bacterial contamination, only a significant positive correlation and of moderate intensity was detected between TSA and SDA ($r_S = 0.565$, $p = 0.022$), revealing that higher bacterial counts in TSA are related with higher fungal counts in SDA. As for fungal contamination, the following significant correlations were detected in a positive sense and with intensity ranging from moderate to strong: i) MEA with fungal counts in VCZ ($r_S = 0.648$, $p = 0.007$); ii) DG18 with ITZ ($r_S = 0.666$, $p = 0.005$) and in VCZ ($r_S = 0.756$, $p = 0.001$). These results revealed that higher fungal counts in MEA are related with higher fungal counts in ITZ and VCZ (Table 5).

Concerning the comparison between the sampling methods, statistically significant differences were detected regarding: i) bacterial counts in TSA (χ^2_{K-W} (2) = 42.461, $p < 0.0001$) and in mean VRBA (χ^2_{K-W} (2) = 42.815, $p < 0.0001$), the swabs having the highest values in both media; ii) fungal counts in MEA mean (χ^2_{K-W} (2) = 27.752, $p < 0.0001$) and in DG18 mean (χ^2_{K-W} (2) = 16.126, $p < 0.0001$), with swabs and settled dust presenting the highest values; iii) fungal resistance counts in SDA (χ^2_{K-W} (2) = 51.149, $p < 0.0001$), in ITZ (χ^2_{K-W} (2) = 6.966, $p < 0.0001$) and in VCZ (χ^2_{K-W} (2) = 15.166, $p < 0.0001$). The swabs were the method with the highest values in SDA, while in ITZ were the swabs and settled dust and in VCZ it was the settled dust that presented the highest values (Fig. S5 - Supplementary material).

4. Discussion

Several waste handling activities, such as waste collection, are physically performed by workers on a daily basis without any kind of mechanization and with reduced protective measures (Nair, 2021). During these activities, bioaerosols are generated and a wide array of microbiological agents (including viable fungi and bacteria, viruses and mycotoxins) and non-biological particles are aerosolized, meaning workers are exposed to risk factors for their health (Pearson et al., 2015; Golwala et al., 2021; Nair, 2021; Szulc et al., 2022). However, exposure to mycotoxins in workplace environments is not commonly recognized, remaining as a non-identified occupational risk factor (Viegas et al., 2020i). Comprehensive assessments of exposure to microbiological and chemical risk factors in waste sorting are, therefore, important to support possible measures to be implemented, as well as to inform and educate workers about potential health risks and precautions to avoid exposure (Szulc et al., 2022).

The optimized sampling and analysis strategy followed in this study

Table 5
Study of the relationship between bacterial contamination (TSA and VRBA), fungal contamination (MEA and DG18) and azole resistance screening (SDA, ITZ, VCZ and PSZ). Results of Spearman's correlation coefficient.

			Bacteria	Fungi		Azole resistance screening			
			VRBA	MEA	DG18	SDA	ITZ	VCZ	PSZ
Swabs	Bacteria	TSA	0.283[a]	−0.028	−0.152	−0.125	−0.140	−0.079	−0.145
		VRBA		0.079	0.100	0.174	−0.216	−0.073	0.082
	Fungi	MEA			0.733[b]	0.392[b]	0.235	0.169	0.158
		DG18				0.485[b]	0.274[a]	0.254[a]	0.212
	Fungal resistance screening	SDA					−0.015	0.046	0.265[a]
		ITZ						0.393[b]	0.192
		VCZ							0.216
Settle dust	Bacteria	TSA	−0.099	0.352[a]	0.506[b]	0.507[b]	0.411[a]	0.233	0.057
		VRBA		−0.022	−0.066	−0.066	0.053	−0.150	0.108
	Fungi	MEA			0.686[b]	0.704[b]	0.657[b]	0.447[b]	0.348[a]
		DG18				0.535**	0.572**	0.376[a]	0.398[a]
	Fungal resistance screening	SDA					0.576[b]	0.586[b]	0.206
		ITZ						0.472[b]	0.501[b]
		VCZ							0.452[b]
EDC	Bacteria	TSA	0.402	−0.393	−0.116	0.565*	0.106	−0.118	−0.062
		VRBA		−0.352	−0.058	0.127	0.004	0.055	0.318
	Fungi	MEA			0.434	0.095	0.032	0.648[b]	0.077
		DG18				0.123	0.666[b]	0.756[b]	0.464
	Fungal resistance screening	SDA					0.029	0.048	−0.329
		ITZ						0.605[a]	0.342
		VCZ							0.356

[a] Correlation is significant at the 0.05 level (2-tailed).
[b] Correlation is significant at the 0.01 level (2-tailed).

unveiled a neglected occupational risk in the waste sorting environment, with detection of mycotoxins in both types of samples types collected from WCT. These results differ from previous studies in which HVAC filters from waste collection trucks were negative for the presence of mycotoxins (Viegas et al., 2017, 2020c). However, in an earlier study developed in the waste management industry from Portugal, by Viegas and colleagues (Viegas et al., 2014), the exposure to aflatoxin B1 was confirmed and exposure to other mycotoxins was reasoned as probable, due to the diverse fungal contamination observed in this workplace environment (Viegas et al., 2015a). Indeed, in occupational settings where exposure to organic dust occurs (e.g., waste management, intensive animal production, farming and many others), workers can also be exposed to mycotoxins (Malik et al., 2014; Straumfors et al., 2014; Viegas et al., 2018e, 2020i).

Exposure to mycotoxins occurs through inhalation and essentially in tasks linked to high exposure to organic dust, in which the dust acts as a carrier of mycotoxins for the respiratory system of workers (Viegas et al., 2018e). In this research, the most frequent (53.1% samples) and diversified (4 mycotoxins) contamination observed in the WCT settled dust may represent a risk of exposure by inhalation, since the dust can be easily resuspended, thus, promoting workers exposure to mycotoxins. It also reinforces the settled dust collection as the best approach to assess mycotoxin contamination of the workplace, by allowing the measurement of several mycotoxins and identification of the occurrence of contamination between cleaning procedures (Halstensen et al., 2006; Tangni and Pussemier, 2007; Viegas et al., 2018e, 2019b). The fact that mycotoxins were only detected in WCT and not in WCS might be due to the higher frequency of waste that is moved by the WCT and lower cleaning frequency that promotes contamination accumulation.

In waste collection, as in other occupational contexts where exposure to organic dust also occurs, the co-exposure to different mycotoxins is the most common scenario (Viegas et al., 2013, 2018a,b,e), with several mycotoxins found in the same type of sample (WCT settled dust, in this research). This multiple exposure is caused by the co-occurrence of different mycotoxigenic fungal species, as well as the capacity of some fungi to produce several mycotoxins simultaneously (Wallin et al., 2015). As is well recognized, adverse health effects from exposure to mycotoxins vary depending on exposure levels and mycotoxin mixtures, being particularly critical in the case of mycotoxin associations with similar effects or mechanism of action, which brings additional challenges in the approach to be followed in risk assessments (Assunção et al., 2016).

EDC sampling is suitable for large-scale epidemiological studies that aim to measure microbial exposure (Madsen et al., 2012; Shorter et al., 2015) by applying molecular tools (Van Cleef et al., 2014; Adams et al., 2015, 2021; Shorter et al., 2015; Bos et al. 2016) or culture based-methods (Ege et al., 2011; Madsen et al., 2012; Viegas et al., 2018d, 2020b,f, 2021b). EDC was not the sampling method presenting the highest fungal counts and diversity in this waste collection research, in contrast with other occupational environments (Viegas et al., 2019a, 2021d,f). Concerning bacteria, EDC sampling presented the highest counts of total bacteria, which maybe explains the lesser fungal contamination of EDC due to competition (Zhu et al., 2003; Mille-Lindblom et al., 2006). These aspects strongly suggest the need to apply a specific sampling strategy for each evaluated occupational environment, since different outcomes can be obtained using the same sampling method.

Regarding fungal diversity, although a lower fungal diversity was observed with DG18 compared to MEA in WCS and in WCT, the same was not observed for *Aspergillus* genera on DG18 with higher number of sections identified in the samples obtained from WCT. This was the expected since DG18 restricts the growth of some fungal species with fast growth rates (mainly from the Mucorales order), thus allowing the identification of otherwise unnoticed toxigenic fungal species, such as *Aspergillus* sp. (Beuchat and Hwang, 1996; Caetano et al., 2018; Viegas et al., 2019a, 2020d, 2021c,d). In light of previous results, MEA or DG18 should be selected by taking into consideration also the *Aspergillus* section targeted, claiming attention for the importance to precisely defining the culture media applied in exposure assessments when assessing *Aspergillus* sp. (Viegas et al., 2021c).

Previous studies conducted in waste sorting and incineration facilities in Portugal have reported the *Aspergillus* genus as the most prevalent on air, surfaces, heating, ventilation and air conditioning filters from forklifters operating in waste sorting plants, and in protection devices used by waste sorting workers (such as filtering respiratory protection devices and mechanic protection gloves) (Viegas et al., 2015a, 2017; 2020d,g). Other assessments in waste sorting showed a significant prevalence of the *Penicillium* genus (Malta-Vacas et al., 2012;

Lehtinen et al., 2013; Pinto et al., 2015). In the current study, besides *Aspergillus* and *Penicillium* genera, *Cladosporium* sp. was also prevalent in WCS and WCT. *Cladosporium* sp. is, in fact, the most prevalent fungus found in Portugal (Aira et al., 2012; Ferro et al., 2018) and this difference can be due the fact that WCT operate mainly outdoors, bringing the contamination inside the WCS.

The *Aspergillus* diversity trend observed in different studies in the waste industry was also observed in this research, with different *Aspergillus* sections identified by culture-based methods (*Fumigati, Nigri, Aspergilli, Candidi, Circumdati* and *Flavi*) and detected by molecular screening (*Fumigati)*. These data corroborate the presence of species/sections with clinical and toxicological relevance, thus indicating harmful fungal contamination in WCS and WCT (AIAH, 2009; APA, 2009; Varga et al., 2015; Viegas et al., 2017, 2020d,e; Sabino, 2020). In WCS *Fumigati* section was not observed, but in WCT this section was the most prevalent in all passive sampling methods, following also the trend of mycotoxins detection. Although the sampling approach in *Aspergillus* sp. exposure assessment can influence the sections found (Viegas et al., 2021c), *Fumigati* prevalence in WCT was the expected due to the previous results obtained in waste management industry in Portugal (Viegas et al., 2014, 2015a,b,2018d,2020c,g, 2021a). As such, these findings corroborate the use of this section as a surrogate of harmful fungal contamination in the waste management industry.

Concerning *Aspergillus* identification (by culture and molecular detection), *Fumigati* was the only *Aspergillus* section detected with both methods, though, in different samples. The observed differences support the utmost importance of using both methods, side by side, in exposure assessments, as supported by previous studies in the waste management industry (Malta-Vacas et al., 2012; Gutarowska et al., 2018; Viegas et al., 2015a,b, 2018d, 2020c; Yimin et al., 2021).

The reduced azole susceptibility of *Aspergillus* sp. from passive sampling has been described in previous assessments of Portuguese workplaces, namely, healthcare facilities (Viegas et al., 2019a) and firefighters' headquarters (Viegas et al., 2021c). Noteworthy, pan-azole-resistant *A. fumigatus* isolates, carrying the TR34/L98H mutation, have been identified in filtering respiratory protection devices used by workers in a Portuguese waste sorting industry (Gonçalves et al., 2021). In the present study, however, no *Aspergillus* sp. was able to grow at tested azole concentrations. As similar sampling and azole screening methods were used, differences from previous studies might be probabilistic given the low number of samples collected (N = 149), compared to previous studies. In fact, a recent national surveillance environmental study of azole-resistant *Aspergillus fumigatus* in Greece, in which 716 samples were collected, unveils percentages of 0.2% of *Aspergillus* isolates, and 1% of *A. fumigatus* species complex isolates, as pan-azole-resistant (itraconazole, voriconazole, isavuconazole and posaconazole MIC >8, >8, >8 and 1 mg/L, respectively) (Siopi et al., 2020). Nevertheless, other filamentous fungi (*Penicillium* sp. and *Fusarium* sp.) associated with human health risks and opportunistic pathogens for immunocompromised hosts (Egbuta et al., 2017) were identified as presenting reduced susceptibility to tested medical azoles.

Azole resistance in the environment is of concern, as it threatens medical therapy, as best described in the treatment failure of invasive fungal infections caused by *A. fumigatus* (Snelders et al., 2012; Verweij et al., 2020). Of note, the significant positive correlation found between fungal growth in itraconazole and voriconazole media might hinder a multi-azole resistance pattern, representing a higher risk of exposure for immunocompromised workers in the assessed setting. Estimating the clinical impact of these results is, however, challenging, since awareness of azole resistance in *Aspergillus* sp. in the environment is scarce in our country. The lack of routine assessment of azole resistance in environmental sampling and of antifungal surveillance initiatives lead to underdiagnosed azole resistance cases. Therefore, hotspots for the development of azole resistance, involving fungal growth in waste containing azole residues (Snelders et al., 2012), must be continuously surveyed in order to list and prioritize major risk factors for fungal resistance selection and enable preventive interventions.

As gliotoxin and other fungal constituents of *Aspergillus* species have been described as exhibiting cytotoxicity (Kamei et al., 2002; Liu et al., 2017), the cell metabolic activity of human lung epithelial (A549) and swine kidney (SK) cells exposed to the collected EDC was assessed in this research by the MTT assay. The minimal or inexistent cytotoxic effects observed are in agreement with the absence of mycotoxins in EDC, and also with previous studies in filtering respiratory protective devices used by waste sorting workers (Viegas et al., 2021f). The IC50 levels were similar, thus, suggesting the EDC as an adequate surrogate for assessing exposure to aerodispersed and inhalable fungal spores.

Finally, the application in this study of the recommended sampling and detection methods for SARS-CoV-2 research (Bertin instruments 2021; Pena et al., 2021) and, consequently, all negative results for SARS-CoV-2 suggest the effectiveness of the implemented measures at the workplace to contain the infection. There is also the positive impact of the high rate of vaccination against COVID-19 in Portugal, since, at the time, the sampled workers were already vaccinated, given that waste collection is considered a frontline and priority sector.

5. Conclusions

In conclusion, our study reveals that a comprehensive sampling approach using active and passive sampling (e.g. settled dust sampling for a representative mycotoxin evaluation) and combined analytic methods (i.e., culture-based and molecular) is an important asset in microbial exposure assessments. Concerning the waste collection exposure scenario, the results of this study unveiled a complex exposure, particularly to fungi and their metabolites. *Aspergillus* section *Fumigati* highlight the significance of targeting this section in the waste management industry as an indicator of occupational health risk. In parallel, this fact associated with the positive correlation between fungal growth in distinct azoles act as a warning for systematic local surveillance of azole resistance in this workplace, as it might be a hotspot for the development of azole resistance. Given that early treatment is critical for a favorable prognostic of azole-resistant aspergillosis, the One Health approach and adequate policies to track both clinical and environmental azole resistance of *Aspergillus* sp. would greatly contribute to identify factors for fungal resistance selection and the implementation of preventive interventions.

Overall, this study results allowed to support preventive and corrective measures in this specific occupational environment, as well as to inform and educate truck drivers about their potential health risks and safety measures to avoid exposure.

Credit author statement

Carla Viegas: Conceptualization; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Roles/Writing - original draft; Writing – review & editing. Pedro Pena: Formal analysis; Methodology; Marta Dias: Formal analysis; Methodology; Bianca Gomes: Formal analysis; Methodology; Renata Cervantes: Formal analysis; Methodology; Elisabete Carolino: Formal analysis; Methodology; Magdalena Twarużek: Formal analysis; Methodology; Ewelina Soszczyńska: Formal analysis; Methodology; Robert Kosicki: Formal analysis; Methodology, Liliana Aranha Caetano: Roles/Writing - original draft; Formal analysis; Methodology; Writing – review & editing. Susana Viegas: Methodology; Roles/Writing - original draft; Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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