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Microbial contamination in firefighter Headquarters': A neglected occupational exposure scenario

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ABSTRACT

One of the occupational environments that need intervention to overcome the lack of information concerning exposure to biological agents is the Firefighter Headquarters' (FFH). This study intends to assess the microbial contamination in Portuguese FFH with a multi-approach protocol for sampling and analyses using active (air samples by impaction and impingement) and passive (surface swabs collected as swabs, settled dust, cleaning cloths and mops, electrostatic dust cloths and identification badges) sampling methods. The fungal contamination was also characterized through molecular detection of toxigenic species, antifungal resistance and mycotoxins profile. Cytotoxicity assessment was included in specific cells line using the MTT assay in order to recognize the possible health effects. It was raised concern regarding the following findings: a) microbial contamination didn't comply with Portuguese IAQ legal requirements in most of the FFH (bacteria load 63.63%, fungal load 45.45%); b) fungal exposure through inhalation underlining a possible risk factor for respiratory diseases; c) prevalence of *Aspergillus* sp. in cleaning materials that might represent a potential source of cross-contamination; d) widespread of *Aspergillus* section *Fumigati* in all the FFH analysed; e) trend of multidrug resistance found among fungi with focus on *Fumigati* isolates; f) detection of several mycotoxins (fumonisin B2, nivalenol, mycophenolic acid and sterigmatocystin) and; g) potential role of *Aspergillus* genus in the cytotoxicity found, particularly in lung cells. The multi-approach on sampling methods (active and passive) and laboratory assays (culture based-methods, molecular tools, mycotoxins detection, cytotoxicity evaluation) improved data findings, enabling a more detailed and accurate risk characterization.

1. Introduction

The Sustainable Development Goals suggested by WHO, and more specifically the goals 3 and 8, emphasizes the need to ensure healthy lives and promote wellbeing and decent work to all, respectively (https://sdgs.un.org/goals). Recently, WHO identified as priority settings for action the workplaces reporting that more than 1.2 million deaths annually (2015 data) are estimated to be caused by occupational risks, and only a small fraction of the global workforce has access to occupational health services (https://www.who.int/tools/compendium-on-health-and-environment/priority-settings-for-action). Thus, there is still a need to intervene and develop actions aiming to improve workplace conditions, being the first steps the risk characterization in the different occupational environments [1,2].

Biological agents exposure was already considered a main health problem in several settings, such as agriculture, waste industry, animal production, medical or veterinary facilities, diagnostic laboratories, plants producing biofuel from rape blossoms, the metallurgical industry, libraries, and even art conservation [3–7]. However, there are some very specific occupational environments that still need a more deeper

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characterization in what concerns exposure to biological agents and their metabolites.

In the context of risk assessment and management of biological agents, recent research has highlighted the significance of comprehending the processes through which airborne fungus cause damage to human health, including fungal cytotoxic effects [8–11]. The assessment of exposure to bioaerosols and biological contaminants and their mixtures with other chemicals is a challenging and dynamic process, and further information on the consequences of combined and repeated exposure to complex mixtures on public health, particularly in occupational contexts, is necessary [12–14].

One of the occupational environments that need intervention to overcome the lack of information concerning exposure to biological agents, is the Firefighters Headquarters (FFH). Previously, a study performed in Portuguese firefighter's ambulances reported several critical aspects: toxigenic fungi with clinical relevance found in ambulances' air, contamination of surfaces increased after cleaning at some sites, MRSA and azole-resistant fungi were detected in ambulances, mycotoxins were detected in mops used for cleaning and electrostatic dust cloths and ambulances' crew presented colonization with MSSA and MRSA [15]. Therefore, a concern regarding the microbiological status in the headquarters was raised and the need of in-depth assessment was identified. In fact, large part of the firefighters' shift is spend in the headquarters, having overnight shifts and waiting for emergency calls [16]. The presence of pathogens is this occupational environment was already reported focusing in bacterial contamination [17–21]. Additionally, bacterial resistance was identified in the surfaces from FFH, where 58% of the sampling locations were MRSA positive, and the firefighters presented colonization with MRSA (37.5%) [19]. More recently, evidence reports the widespread of *Aspergillus* genera regarding this occupational environment, with section *Fumigati* being present in all FFH. Also, the presence of mycobiota potentially resistant to azoles was observed, emphasizing the need of corrective and preventive measures [2].

This study intends to assess the microbial contamination (fungi, bacteria and SARS-CoV-2 due to the pandemic crises ongoing during the sampling campaign) in Portuguese FFH with a multi-approach protocol for sampling and analyses using active (air samples by impaction and impingement) and passive (surface swabs collected as swabs, settled dust, cleaning cloths and mops, electrostatic dust cloths and identification badges) sampling methods. The fungal contamination in the assessed headquarters was also characterized through molecular detection of toxigenic species, antifungal resistance and mycotoxins profile. Cytotoxicity assessment was included in specific cells line using the MTT assay in order to recognize the possible health effects related with FFH exposure scenario.

2. Materials and methods

2.1. Firefighter headquarters' selection and characterization

Eleven firefighter headquarters in the district of Lisbon were selected for study between September 2020 and May 2021, as part of a funded research study to characterize the occupational exposure of firefighters to microbial contamination. Common tasks developed by the firefighters in these headquarters included civil protection and assistance in situations of fire, floods and landslides, accidents and catastrophes, as well as rescue of pets and wild animal. About 800 firefighters in the assessed headquarters were usually enrolled in these activities.

In each firefighter headquarter, a walkthrough survey and checklist were used to gather information about the cleaning procedures performed, the number of firefighters who normally occupy the installations, the ventilation system adopted and other characteristics than can influence microbial growth and dissemination (Table 1). Regarding the cleaning procedures, all areas of the headquarters were cleaned and disinfected once a day, and are performed by an external company. Several areas were assessed in all headquarters, namely: dormitory, locker rooms, kitchen, canteen, bar, living room, administrative room, reception and gym. The sampling campaign was performed during one day period at the same time of a normal working day. In most FFH the ventilation was only provided through the open of windows and doors (Table 1).

2.2. Sampling campaign

The sampling methods and number of samples collected per FFH are depicted in Table 1.

The air samples instruments used for the air impaction method in this study were Millipore air tester (Millipore, Billerica, MA, USA) and Andersen six-stage air sampler, with four different culture media to selectively collecting fungi and bacteria. Samples of indoor air (and outdoor air, as reference) were collected from each previously defined area in the headquarters, by collecting 250 L at a flow rate of 140 L/min with the Millipore air tester, or at a flow rate of 28.3 L/min, for 9 min in each culture medium with the Andersen air sampler, according to manufacturer's instructions and reported method [22].

For the purposes of mycotoxins' assessment and SARS-CoV-2 detection, a Coriolis μ air sampler (Bertin Technologies, Montigny-le-Bretonneux, France) was used at the airflow rate of 300 L min− 1 during 2 min (Impingement method), with air samples pumped into vials with 10 ml sterile phosphate-buffered saline (PBS - pH 7.4) with 0.05% Triton X100.

Electrostatic dust collectors (EDC), floor surface swabs, settled dust, cloths and mops used in cleaning procedures, and identification badges from firefighters' uniforms were used as passive sampling strategy. Briefly: EDC were placed in the sampling areas 1.5 m above the ground for 30 days; floor surfaces were swabbed using a 10 × 10cm square stencil (disinfected between each sampling with a 70% alcohol solution); a common vacuum cleaner (and respective filter) were used to collect settled dust, analysed as composite sample [2,22]; cloths, mops and identification badges were collected. All samples were maintained refrigerated (0-4 °C) in sterilized bags prior to analysis [15].

2.3. Sample wash and characterization of viable microbiota

Passive samples were washed with 0.1% Tween 80 saline (0.9% NaCl) solution (250 rpm, 30 min), as follows: 1 mL solution for floor surface swabs; 10 mL solution for identification badges and for each settled dust filter (2 cm^2), cleaning cloths and mops; 20 mL solution for (weighted) EDC; 9.1 mL solution for 1 g of a composite settled dust sample from all FFH [15].

Malt extract agar (MEA) supplemented with chloramphenicol (0.05%), dichloran-glycerol agar (DG18), tryptic soy agar (TSA) supplemented with nystatin (0.2%), and Violet Red bile agar (VRBA) were used for fungi (MEA and DG18, 27 °C, 5–7 days), mesophilic (TSA, 30 °C, 7 days) and Gram-negative (VRBA, 35 °C, 7 days) bacteria selectivity. Microbiota quantification was determined as colony-forming units (CFU) and CFU concentration ($CFU.m^{-3}/m^{-2}/m^{-2}.day^{-1}/g^{-1}$) after plates incubation. Morphological identification of fungal species was carried out through notation of macro and microscopic characteristics [23] by an expert mycologist.

2.4. Antifungal susceptibility testing

The screening of azole-resistant fungi was firstly done by seeding 150 μL of the extracts of all passive samples except swabs (N = 289) on Sabouraud dextrose agar (SDA) (Frilabo, Maia, Portugal) supplemented with 4 mg/L itraconazole (ITZ), 2 mg/L voriconazole (VCZ), or 0.5 mg/L posaconazole (PSZ), adapted from EUCAST guidelines (Arendrup et al., 2013; The European Committee on Antimicrobial Susceptibility Testing, 2020). Controls were *A. fumigatus* reference strain (ATCC 204305) as negative control, and pan-azole-resistant *A. fumigatus* strain as positive

Table 1
Ventilation and environmental characteristics of, and sampling methods used in, firefighter headquarters' (FFH) (adopted from [2].

FHH	Type of ventilation	Building number of years	Number of occupants per shift	Air samples methods		Passive sampling methods							Observations
				Air impaction	Impinger	Surface swabs	Filters	Cloths	Mops	EDC	Identification badges	Settled dust	
1	Natural	>100 years	50	112	15	13	13	7	3	13	17	1	Cracks on the walls / old appearance 
2	Natural	>100 years	12	68	9	8	8	2	1	8	4	1	
3	Natural	37	30	76	10	9	9	3	1	6	5	1	Visible damp spots on walls, ceiling or floor 
4	Natural	20	11	60	8	7	7	2	2	6	6	1	Visible damp spots on walls, ceiling or floor; 
5	Natural	>100 years	20	72	10	9	9	1	1	9	5	1	
6	Natural	>100 years	16	76	10	9	9	2	1	7	5	1	Walls and ceiling with infiltrations 
7	Natural	2	7	52	7	6	6	1	1	6	5	1	Visible damp spots
8	Natural	>100 years	16	68	9	8	8	2	1	7	5	1	–
9	Natural	62	20	60	8	7	7	2	1	7	5	1	Visible damp spot on wall
10	Natural	30	20	64	8	8	8	1	1	7	5	1	Visible damp spot
11	Artificial	4	7	52	7	6	6	2	1	6	5	1	–

control, both provided by National Health Institute Doctor Ricardo Jorge, IP. After incubation during 4 days at 27 °C, identification was performed as previously described for fungal assessment [2].

Aspergillus sp. isolates were recovered and subcultured as previously described [24]. The antifungal susceptibility profiles were determined with E-test gradient strips of voriconazole, posaconazole, itraconazole, and amphotericin B (AMB) (Liofilchem) with a concentration gradient ranging from 0.002 to 32 μg/ml for each drug. According to the manufacturer's instructions, each 140-mm petri plate containing solidified RPMI 1640 medium with 2% dextrose (Liofilchem) medium was inoculated with 400 μl of the respective *Aspergillus* sp. inoculum. Plates were incubated at 35 °C, and MICs were determined following incubation times of 24 and 48 h. The E-test MIC was the lowest drug concentration at which the border of the elliptical inhibition intercepted the scale on the antifungal strip.

2.5. Sampling and molecular detection of SARS-CoV-2 and targeted fungal sections

Surface samples were collected by swabbing the areas of each sampling site, using sterile cotton swabs moistened in Buffer NVL (SARS-COV-2 assessment) or sterilized water (fungi and bacteria assessment). A 10 cm × 10 cm square stencil, disinfected between samplings with a 70% alcohol solution was used (ISO 18593: 2004) to allow quantification. On some surfaces with common characteristics, such as surfaces material and cleaning procedures, composite samples were performed (swabbing different surfaces with the same swab) [25]. 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.

The molecular detection of *Aspergillus* sections (Viegas et al., 2021e) was carried out by Real Time PCR (qPCR) in all passive samples' extracts (8.8 ml) except surface swabs. Briefly, fungal DNA extracted from the samples with ZR Fungal/Bacterial DNA MiniPrep Kit (Zymo Research, Irvine, USA)) was analysed in 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 total volume of 20 μl. Amplification was done by a three-step PCR, including 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.

As controls, a non-template control and DNA from a reference as positive control (kindly conceded by the Reference Unit for Parasitic and Fungal Infections, Department of Infectious Diseases, National Health Institute Doctor Ricardo Jorge, IP) were sequenced for ITS, B-tubulin, and Calmodulin.

2.6. Mycotoxins analysis

A total of 360 samples were screened for mycotoxins presence (92 air samples collected by Coriolis air sampler, 9 settled dust samples collected by vacuuming, 90 filters used on the vacuum cleaner, 62 EDC, 25 cleaning cloths, 14 mops and 68 uniform name tags). Air samples (100 μl) were directly diluted 1:7 (v/v) with a 1:1 mixture of water and extraction solvent (acetonitrile (ACN): water (H2O): acetic acid (AcOH) (79:20:1)). HVAC filter samples (0.2 g) were extracted with 2.0 ml of ACN: H2O: AcOH (79:20:1) for 60 min. Raw extracts were diluted with the same amount of water, mixed, centrifuged and injected into the LC-MS/MS system. Detection of mycotoxins was carried out using high performance liquid chromatograph (HPLC) Nexera (Shimadzu, Tokyo, Japan) with a mass spectrometry detector API 4000 (Sciex, Foster City, CA, USA). Mycotoxins were separated on a chromatographic column Gemini NX-C18 (150 × 4.6 mm, 3 μm) (Phenomenex, Torrance, CA, USA); mobile phase (A: water + 5 mM ammonium acetate + 1% acetic acid, B: methanol + 5 mM ammonium acetate + 1% acetic acid) mobile phase flow rate: 0.75 ml/min, injection volume: 7 μl.

The mycotoxins concentration was calculated using external calibration. Several mycotoxins were analysed, namely: patulin, nivalenol, deoxynivalenol-3-glucoside, deoxynivalenol, fusarenon-X, α-zearalanol, β-zearalanol, β-zearalenol, α-zearalenol, zearalanone, zearalenone, T-2 tetraol, deepoxydeoxynivalenol, neosolaniol, 15-acetyldeoxynivalenol, 3-acetyldeoxynivalenol, monoacetoxyscirpenol, diacetoxyscirpenol, aflatoxin M_1, aflatoxin B_1, aflatoxin B_2, aflatoxin G_1, aflatoxin G_2, fumonisin B_1, fumonisin B_2, fumonisin B_3, T-2 triol, roquefortine C, sterigmatocystin, griseofulvin, T-2 toxin, HT-2 toxin, ochratoxin A, ochratoxin B, mycophenolic acid, mevinolin. The Limits of Detection (LOD) obtained for each mycotoxin with the analytical method used are presented in Supplementary material (Table S1).

2.7. Determination of samples' IC50

Coriolis air, filters, settled dust, EDC, identification badges, mops and cleaning cloths samples were prepared by two-fold serial dilutions and used to evaluate the metabolic activity of human lung epithelial (A549) and swine kidney (SK) cells using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [12,26]. Briefly, 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). After detachment from the vessels with 0.25% (w/v) Trypsin 0.53 mM EDTA, cells were suspended in the culture medium. Cell suspensions were counted using a Scepter™ 2.0 Cell Counter (Merck) for densities of $2{,}5 \times 10^5$ cells/ml. Cell suspensions (100 μl) were then incubated with the test samples in a 96-well plate for 48 h at 5% CO2, 37 °C, and humid atmosphere. The cytotoxicity level was measured at 510 nm (ELISA LEDETECT 96, biomed Dr. Wieser GmbH; MikroWin 2013SC software). The lowest concentration dropping absorption to <50% of cell metabolic activity (IC50) was considered the threshold toxicity level.

2.8. Statistical analysis

Data were analysed using SPSS statistical software, V26.0 for Windows. The results were considered significant at the 5% significance level. To test data normality, the Shapiro-Wilk test (n's ≤ 50) or the Kolmogorov-Smirnov test (n's > 50) was used. To compare the microbial contamination through sampling method, and *Aspergillus* section *Fumigati* molecular detection in all matrices, the Kruskal-Wallis test was used, since the assumption of normality was not verified. To study the relationship between sampling method and culture media in fungal and bacteria counts the Spearman's correlation coefficient was used, since the normality assumption was not verified. To assess species diversity, Simpson and Shannon indices, given by $\text{Shannon Index (H)} = -\sum_{i=1}^{s} p_i \ln(p_i)$ and $\text{Simpson Index (D)} = \frac{1}{\sum_{i=1}^{s} p_i^2}$, were used, where p_i is the proportion (n_i/n) of individuals of one particular species found (n_i) divided by the total number of individuals found (n).

3. Results

3.1. Viable bacterial contamination

Total bacteria load (TSA) in indoor sites ranged from 6.4×10^3 to 2.8×10^4 to CFU.m^{-3} in Andersen six stage air samples (Supplementary material - Fig. S1). In air samples from Millipore total bacteria vary from 1.2×10^3 to 2.2×10^4 CFU m^{-3}. Gram-negative bacteria load (VRBA) ranged from 0 to 3.6×10^4 in Andersen and from 4 to 1.6×10^2 CFU m^{-3} in Millipore (Supplementary material - Fig. S1).

Floor surface samples presented the highest median value for bacterial contamination (1.2×10^6 CFU m^{-2}), while Gram-negative counts ranged from 0 to 2.7×10^4 CFU m^{-2}. Concerning filters, bacteria counts ranged between 1.5×10^3 to 3.0×10^5 CFU m^{-2} and Gram-negative had the highest value among the matrices (4.5×10^5 CFU m^{-2}). Cloths and mops used for cleaning routine had similar values of total bacterial, ranging from 5.0×10^2 to 5.5×10^5 CFU m^{-2} and from 5.0×10^2 to 1.9×10^5 CFU m^{-2}, respectively. Gram-negative bacteria vary from 0 to 4.2×10^5 CFU m^{-2} in cleaning cloths and from 0 to 5.5×10^5 CFU m^{-2} in mops (Supplementary material - Fig. S1).

Lowest values of total bacteria were obtained from uniform name tags, ranging from 1.5×10^3 to 3.4×10^4 CFU m^{-2} and Gram-negative bacteria from 0 to 5.0×10^2 CFU m^{-2}. Total bacterial in EDCs samples ranged between 2.1×10^1 to 5×10^2 CFU m^{-2}.day^{-1} and Gram-negative bacteria from 0 to 8.1×10^2 CFU m^{-2}.day^{-1}. In settled dust samples, total bacteria and Gram-negative values ranged from 4 to 5.6×10^2 CFU g^{-1} (Supplementary material - Fig. S1).

3.2. Viable fungal contamination

Fungal Load in indoor sites ranged from 7.6×10^2 to 6.5×10^4 CFU m^{-3} on MEA and from 7.4×10^2 to 2.0×10^4 CFU m^{-3} on DG18, in Millipore air samples. Fungal counts from Andersen six-stage vary between 7.4×10^3 to 7.6×10^4 CFU m^{-3} and from 1.5×10^3 to 2.3×10^4 CFU m^{-3} on MEA and DG18 respectively (Supplementary material - Fig. S2).

Floor surface swabs had the highest fungal contamination, ranging from 7.5×10^3 CFU m^{-2} to 1.2×10^6 CFU m^{-2} on MEA and from 3×10^3 CFU m^{-2} to 1.3×10^6 CFU m^{-2} on DG18, followed by filters, with values ranging between 2.1×10^4 to 1.6×10^6 CFU m^{-2} on MEA and from 2.7×10^4 to 8.7×10^5 CFU m^{-2} on DG18. Name tags presented a range of fungal counts between 2×10^3 to 9.5×10^3 CFU m^{-2} on MEA and from 0 to 1.4×10^4 on DG18 (Supplementary material - Fig. S2).

Regarding mops and cleaning cloths lowest values were obtained (MEA: 5×10^2 to 1.2×10^4, DG18: 0 to 4×10^3 CFU m^{-2}; MEA: 0 to 9.5×10^3, DG18: 0 to 9.5×10^3 CFU m^{-2} respectively). Fungal results on EDCs ranged from 4.7×10^1 to 2×10^3 on MEA and from 1.4×10^1 to 2.1×10^3 CFU m^{-2}.day^{-1} on DG18. Settled dust counts vary from 12 to 3.6×10^3 on MEA, and from 8 to 7.9×10^2 CFU g^{-1} on DG18 (Supplementary material - Fig. S2).

Concerning fungal distribution per sampling method, the highest fungal diversity was obtained from active sampling through Andersen six-stage, where 27 species were observed on MEA and 22 species on DG18 (Fig. 1).

Through the air sampled from Millipore, 19 species were identified on both MEA and DG18. Among passive methodologies, the highest fungal diversity was associated to EDC samples with 18 species being detected on MEA and 15 species on DG18. In samples from filters, 13 species were reported on MEA and 10 species on DG18, followed by swabs (MEA: 10 species; DG18: 7 species) and settled dust from filters (MEA: 7 species; DG18: 12 species). Lower fungal diversity was observed on mops (MEA:5 species; DG18:5 species), cleaning cloths (MEA:5 species; DG18:4 species) and name tags (MEA:4 species; DG18:4 species).

The most common species in air samples obtained by Andersen six-stage device was *Chrysonilia sitophila* (51.85% MEA; 52.00% DG18). In Millipore on DG18 (47.77%) *C. sitophila* was also the most found, while on MEA *Rhizopus* sp. (40.07%) was the prevalent.

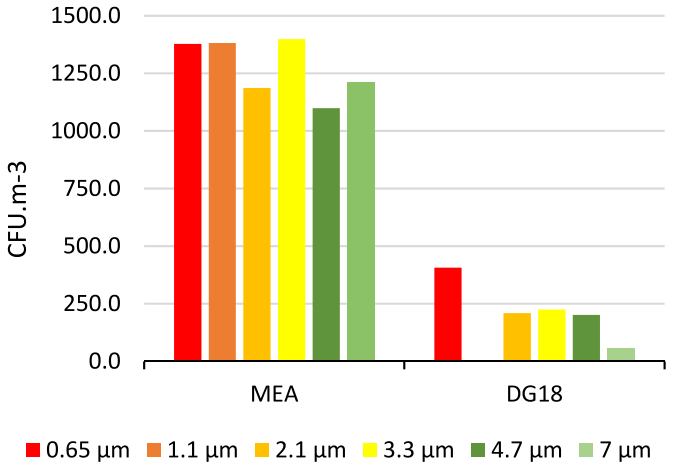

Fig. 1. Fungal distribution from air samples collected by Andersen Six-stage inoculated in MEA and DG18 (samples were obtained from the FFH with the highest fungal counts).

Concerning samples from passive methods, *Cladosporium* sp. was the most frequent genera on filters (72.68% MEA; 59.97% DG18) and EDCs (62.89% MEA; 86.99% DG18), followed by floor surface swabs (62.97% MEA; 67.74% DG18) and settled dust samples from DG18 (59.99%). *Penicillium* sp. was the prevalent genera in settled dust samples on MEA (68.56%). Also, similar results regarding the genera in DG18 were obtained in mops (47.17% MEA; 46.88% DG18), in cleaning cloths (41.67% MEA; 59.73%) and in name tags samples (38.46%). While on MEA, C.*sitophila* was the most frequent (54.95%) (Table 2).

Regarding *Aspergillus* sp., the highest value of the genera was obtained in DG18 (2.20%) comparatively with MEA (1.52%). The burden of *Aspergillus* in air samples was higher in samples from Andersen six-stage (0.12% MEA, 1.51% DG18) when compared to Millipore (0.11% MEA, 0.78% DG18) (Fig. 2).

Among samples from passive methods, in DG18, the most contaminated matrix with *Aspergillus* sp. were mops (15.63%) and cleaning cloths (7.08%). The genus was also present in swabs (2.36%), EDC (2.16%), filters (1.91%) and settled dust (0.90%). The genus was not identified on name tag samples. On MEA, trough passive methods, the matrices with the highest values of the genera were filters (3.37%), followed by cleaning cloths (1.67%), EDCs (0.35%) and swabs (0.22%). The genus was not identified in name tags, mops and settled dust samples (Fig. 2).

On DG18, 8 *Aspergillus* sections were identified, namely *Fumigati* (33.01%), *Nidulantes* (29.38%), *Candidi* (20.22%), *Aspergilli* (7.68%), *Circumdati* (6.11%), *Flavi* (3.23%), *Restricti* (0.27%) and *Nigri* (0.10%). While on MEA 7 species were reported, as follows: sections *Nidulantes* (57.31%), *Circumdati* (32.49%), *Candidi* (7.16%), *Nigri* (1.46%), *Flavi* (1.25%), *Fumigati* (0.33%) and *Aspergilli* (0.003%).

Regarding sections identification, in air samples from Andersen six-stage, the section *Nidulantes* was predominant on MEA (45.04%), while the section *Candidi* was frequent on DG18 (30.57%). *Aspergillus* section *Nidulantes* was detected in all the six stages, having the highest prevalence (44.85%) on stage 4 (2.1 µm). On stage 5 (1.1 µm) the section had a prevalence of 21.53% and on stage 6 (0.65 µm) a prevalence of 10.76%. Section *Candidi* was equally detected in all the six stages, having the highest values on stages 4 (64.20%) and stage 5 (17.28%). In Millipore air samples, 4 sections were detected on MEA (79.77% *Fumigati;* 15.16% *Nidulantes*; 2.53% *Aspergilli;* 2.53% *Nigri*), and 6 sections were identified in DG18 (36.54% *Nigri;* 24.43% *Aspergilli*; 14.25% *Candidi;* 14.25% *Circumdati,* 7.38% *Nidulantes,* 3.05% *Flavi)* (Supplementary material – Fig. S3).

Concerning sections distribution on EDCs, 4 *Aspergillus* sections were

Table 2
Fungal distribution per sampling method.

SAMPLE	MEA			DG18		
	Fungi	CFU. $m^{-3}/m^{-2}/g^{-1}$*/$CFU.m^{-2}.day^{-1}$	%	Fungi	CFU. $m^{-3}/m^{-2}/g^{-1}$/*$CFU.m^{-2}.day^{-1}$	%
ANDERSEN	*C. sitophila*	210384.8	51.85	*C. sitophila*	35857.87	52.00
	Chrysosporium	65665.47	16.18	*Cladosporium* sp.	21405.6	21.04
	Penicillium sp.	85360.32	21.04	*Penicillium* sp.	10117.77	14.67
	Aspergillus sp.	486	0.12	*Aspergillus* sp.	1040	1.51
	Other species	43871.22	10.82	Other species	533.9585	0.77
MILLIPORE	*Rhizopus* sp.	59131	40.07	*C. sitophila*	24370	47.77
	C. sitophila	25897	17.55	*Cladosporium* sp.	14509.3	28.44
	Cladosporium sp.	21451	14.54	*Penicillium* sp.	9329.342	18.29
	Aspergillus sp.	158.16	0.11	*Aspergillus* sp.	393	0.77
	Other species	40920,84	27.73	Other species	2416	4.74
EDC	*Cladosporium* sp.	5190.3	72.68	*Cladosporium* sp.	4348.1	59.97
	Penicillium sp.	923.47	12.93	*Penicillium* sp.	1811.3	24.98
	Aspergillus sp.	25.074	0.35	*Aspergillus* sp.	2770.3	2.16
	Other species	1002.5	14.04	Other species	4640.13	12.89
CLEANING CLOTHS	*Penicillium* sp.	12500	41.67	*Penicillium* sp.	59.73	59.43
	C. sitophila	11500	38.33	*Cladosporium* sp.	26.42	26.41
	Cladosporium sp.	5000	16.667	*Aspergillus* sp.	7.08	7.08
	Aspergillus sp.	500	1.67	Other species	7.08	7.08
	Other species	500	1.67			
MOPS	*Penicillium* sp.	12500	47.17	*Penicillium* sp.	7500	46.88
	C. sitophila	7000	26.42	*Cladosporium* sp.	5000	31.25
	Cladosporium sp.	4000	15.09	*Aspergillus* sp. Other species	2500	15.63
	Other species	3000	11.32		1000	6.25
NAME TAGS	*C. sitophila*	25000	54.95	*Penicillium* sp.	12500	38.46
	Cladosporium sp.	15500	34.07	*C. sitophila*	11000	33.85
	Penicillium sp.	5000	10.99	*Cladosporium* sp.	8500	26.15
				Other species	500	1.54
FILTERS	*Cladosporium* sp.	2395100	62.89	*Cladosporium* sp.	2393500	86.99
	Penicillium sp.	691600	18.16	*Penicillium* sp.	276500	10.05
	C. sitophila	570000	14.97	*Aspergillus* sp.	52505	1.91
	Aspergillus sp.	128500	3.37	*C. sitophila*	29000	1.05
	Other species	23000	0.60			
SETTLED DUST	*Penicillium* sp.	4454	68.56	*Cladosporium* sp.	1716	56.99
	Cladosporium sp.	1677	25.81	*Penicillium* sp.	1212.4	40.27
	Other species	365.5	5.63	*Aspergillus* sp.	27	0.90
				Other species	55.5	1.84
SWABS	*Cladosporium* sp.	2879500	62.97	*Cladosporium* sp.	2866500	67.74
	Penicillium sp.	854500	18.69	*Penicillium* sp.	713000	16.85
	Phoma sp.	590000	12.90	*C. sitophila*	552000	13.04
	Aspergillus sp.	10000	0.22	*Aspergillus* sp.	100000	2.36
	Other species	238500	5.22	Other species	55.5	0.001

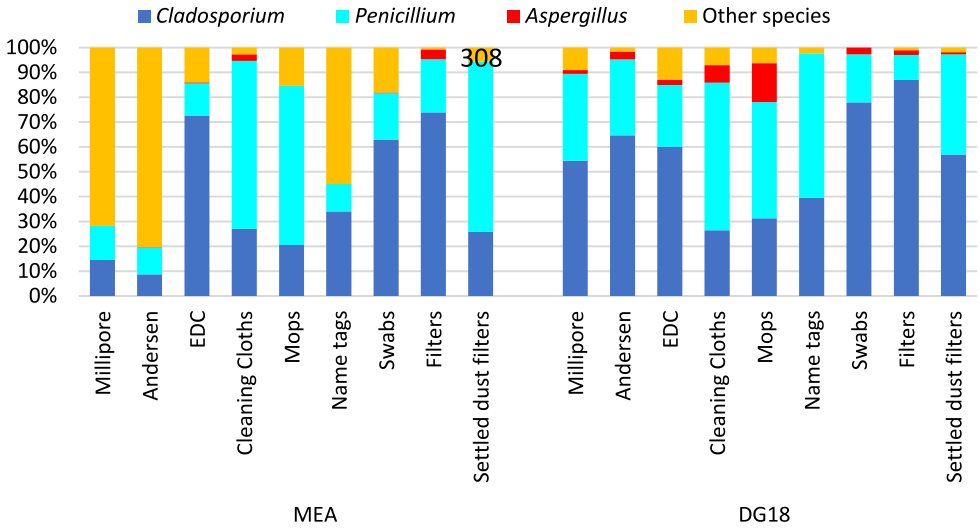

Fig. 2. Fungal species distribution per matrix in MEA and DG18.

detected on MEA (28.57% *Fumigati, Nidulantes, Flavi* and 14.29% *Circumdati*) and 6 sections on DG18 (34.29% *Circumdati;* 29.75% *Aspergilli*; 13.73% *Flavi;* 10.78% *Candidi;* 9.16% *Restricti* and 2.28% *Nidulantes*). On mops the sections *Candidi* (60%) and *Nidulantes* (40%) were prevalent on DG18. In cleaning cloths the section *Nigri* was dominant (100%). In contrast, to the prevalence of section *Flavi* in DG18 (100%), four sections were identified in filter samples on MEA (62.26% *Nidulantes*, 35.41% *Circumdati,* 1.17% *Flavi* and 1.17% *Nigri)* and 6 sections were detected on DG18 (44.76% *Fumigati,* 20.95% *Aspergilli*, 15.24% *Circumdati,* 12.39% *Nidulantes,* 5.71% *Flavi* and 0.95% *Candidi*). *Aspergillus* section *Aspergilli* was prevalent on floor surface samples (100%), while 3 sections were reported in DG18 (40% *Nidulantes*, 30% *Fumigati* and 30% *Candidi*). In settled dust, 2 sections were detected in DG18 (96.30% *Nigri* and 3.70% *Flavi)*.

Regarding the diversity of species found using the active sampling method with higher diversity obtained (air samples collected by Anderson device) and considering the culture media with higher number of species (MEA), it was found that the living room (H = 1.698; D = 4.592), the locker room (H = 1.473; 4.038) and the canteen (H = 1.404; D = 3.620) were the sampling areas in which greater diversity was detected, as they were the ones with the highest values of Shannon's and Simpson's indices (Table S2 - Supplementary material).

Using the EDC (passive sampling method wither higher number of different species), and considering the MEA as well, the sampling areas that presented the greatest diversity of species were the Kitchen (H = 1.251, D = 2.654), the administrative room (H = 1.125, D = 2.292) and the canteen (H = 1.125, D = 2.213) (Table S3 - Supplementary material).

3.3. Fungal diversity in azole-supplemented media

The screening of antifungal resistance in passive samples (EDC, cleaning cloths, mops, uniform name tags, filters, and settled dust) revealed a total of 1.1x10^7 colony-forming units per gram (CFU/g), of which, 4.2x10^6 were observed in SDA (7.1x10 average per sample type), 1.6x10^6 in ITZ (2.6x10^5 average per sample type), 3.9x10^6 in VCZ (6.6x10^5 average per sample type), and 1.6x10^6 in PSZ (2.7x10^5 average per sample type). In SDA, a 36-fold higher burden of fungi was obtained in EDC and filters (4.1x10^6) when compared with all other sampling devices (1.2x10^5) (Table 3).

The most frequently observed fungal genera was *Cladosporium* (44.0%–89.4%) (Table X). The *Cladosporium* distribution (CFU/g) in azole-supplemented media was higher in Filters, as follows: 1,573,000 SDA; 1,371,500 ITZ; 2,609,500 VCZ; 1,203,000 PSZ (Table 3).

At lower frequency (0.1%–1.1%), *Aspergillus* genus was also found (Table 3). The *Aspergillus* distribution (CFU/g) in azole-supplemented media was higher in Filters (395,000 SDA; 500 ITZ) and in EDC (2,637 SDA; 1,062 ITZ; 3,609 VCZ). Regarding *Aspergillus* diversity, six different sections were found, including section *Fumigati* in ITZ and VCZ media (Fig. 3).

3.4. Aspergillus section Fumigati isolates susceptibility profile

The 14 isolates identified as *Aspergillus section Fumigati* were tested for 24 h and 48 h using E-test to evaluate their antifungal susceptibility (MIC) to azoles. The distribution of isolates at 48 h is detailed in Table 4.

3.5. Detection of SARS-CoV-2 and targeted fungal sections

SARS-CoV-2 was not detected in any of the environmental samples collected. Regarding *Aspergillus* section *Fumigati,* it was detected in 146 samples (38.52%; 146 out of 379 samples). Among the 146, 61 (47.8%) were in settled dust samples, 60 (41.1%) in filter samples, 4 (2.47%) in uniform rank samples, 7 (4.8%) in mop samples and 14 (9.6%) in cleaning cloths samples. Concerning *Aspergillus* sections *Nidulantes and Circumdati* both were detected in different settled dust samples (0.26%, 1 out of 379) (Supplementary material - Table S4).

3.6. Mycotoxins contamination

Fumonisin B2 was the mycotoxin most detected in the types of samples considered and it was the only mycotoxin detected in the liquid samples collected through the Coriolis equipment and involving also the tags with the uniform names. Only in the settled dust, cleaning cloths and mops this mycotoxin was not detected. In the filters collected it was detected in 3 samples (3.4%) with values ranging <9 (LOQ) to 9.7 ng/g. In EDCs was detected in 5 samples (8.1%) with values ranging from <9 to 10.4 ng/g. In the case of liquid samples, in 26 samples (37.7%) was detected fumonisin B2 with values ranging <6 (LOQ) and 6.2 ng/g.

Additionally, other mycotoxin was detected in EDCs samples, namely nivalenol that was detected in one sample with a value below the LOQ (14 ng/g). Also in filters from the settled dust a different mycotoxin was identified in one sample - mycophenolic acid - with a value below the LOQ (10 ng/g).

In the settled dust, cleaning cloths and mops the mycotoxins detected were mycophenolic acid in 2 samples with values below the LOQ (20 ng/g) and sterigmatocystin in only 1 sample with value also below the LoQ (6 ng/g).

3.7. Cytotoxicity evaluation

The cytotoxic effects of samples from passive and active sampling methods are presented in Table 5. The IC50 of samples from passive sampling methods ranged from 1.25 mm^2/ml to 20 mm^2/ml, with mops revealing a more expressive cytotoxic effect, followed by cleaning cloths. No cytotoxicity was found in filter samples. In settled dust, IC50 values ranged from 31.25 mg/ml to 500 mg/ml. Regarding air samples, IC50 ranged from 12.5 μl/ml to 50 μl/ml.

3.8. Comparisons and correlation analysis

Concerning microbial contamination through each sampling

Table 3
Fungal distribution per azole-screening media.

Fungi	SDA		ITZ		VCZ		PSZ	
	CFU. m^{-2}/CFU. m^{-2}. day^{-1}/ g^{-1}	%	CFU. m^{-2}/CFU. m^{-2}. day^{-1}/ g^{-1}	%	CFU. m^{-2}/CFU. m^{-2}. day^{-1}/ g^{-1}	%	CFU. m^{-2}/CFU. m^{-2}. day^{-1}/ g^{-1}	%
Aspergillus sp.	44659	1.1	1562	0.1	3609	0.1	0	0.0
C. sitophila	1233848	29.1	89135	5.7	417537	10.6	253813	15.5
Chrysosporium sp.	0	0.0	500	0.0	0	0.0	0	0.0
Cladosporium sp.	1863690	44.0	1396212	89.4	2685930	68.0	1235140	75.3
Penicillium sp.	1063125	25.1	70692	4.5	791946	20.1	145296	8.9
Rhizopus sp.	0	0.0	0	0.0	18000	0.5	0	0.0
Other species	28710	0.7	4510	0.3	32830	0.8	5947	0.4
Total	4234032	100	1562611	100	3949853	100	1640195	100

Fig. 3. *Aspergillus* sections and respective frequency (%) in passive samples, in: a) SDA; b) ITZ; c) VCZ.

Table 4
Antifungal susceptibility of the *Aspergillus* section *Fumigati* isolates collected from different sampling sources in the FFH environment.

Sampling	Device	MIC ITZ (μg/ml)	MIC VCZ (μg/ml)	MIC PSZ (μg/ml)	MIC AMB (μg/ml)
Active (Air)	Andersen	1.5	0.94	0.19	0.19
	Andersen	0.19	0.023	0.08	≤0.002
	Andersen	0.125	0.047	0.094	1
	Andersen	0.75	0.023	0.094	2
	Andersen	≤0.002	≤0.002	≤0.002	≤0.002
	Andersen	0.75	0.047	0.032	0.75
	Andersen	0.047	0.012	0.023	≤0.002
Passive	Mop	8	3	1	0.19
	EDC	1	0.019	0.19	≤0.002
	EDC	2	0.032	0.47	0.25
	EDC	6	2	0.75	0.25
	EDC	8	≤0.002	≤0.002	≤0.002
	EDC	0.25	0.5	1	0.75
	Filter	2	0.125	0.19	≤0.002

method, statistically significant differences were detected between filters and cleaning cloths, mops and uniform name tags in i) MEA ($\chi^2_{K-W}(3) = 28.570$, p = 0.000); ii) DG18 ($\chi^2_{K-W}(3) = 24.789$, p = 0.000); iii) ITZ ($\chi^2_{K-W}(3) = 28.121$, p = 0.000); iv) VOZ ($\chi^2_{K-W}(3) = 22.078$, p = 0.000); v) POZ ($\chi^2_{K-W}(3) = 26.295$, p = 0.000). In all cases filtering was the sampling method presenting the highest values (Supplementary material - Fig. S4).

Considering molecular detection of *Aspergillus* genera statistically significant differences were identified for detection of section *Fumigati* in the matrices ($\chi^2_{K-W}(4) = 12.686$, p = 0.041). EDC were the ones with the highest values, in contrast to the results obtained from name tags (Supplementary material - Fig. S5).

In the filters, only significant correlation was detected between fungal counts in ITZ and VOZ ($r_S = 0.755$ p = 0.007), revealing that higher values of fungal counts in ITZ are related to higher values of fungal counts in VOZ.

Significant correlations were obtained between sampling method (Millipore; EDC; cleaning cloths; mops; settled dust; swabs; name tags) and the culture media used for bacterial and fungal counts. No significant correlations were obtained for filters (Table 6).

Regarding air samples from Millipore, statistically significant correlations were detected between bacteria counts in TSA and fungal counts in MEA ($r_S = 0{,}618$ p = 0.043), meaning that higher bacteria values are associated to higher fungal counts (Table 6).

In case of EDC, statistically significant correlations were detected between: i) bacteria counts in TSA and fungal counts in MEA ($r_S = 0.633$, p = 0.036) and DG18 ($r_S = 0.629$, p = 0.038); ii) Fungal counts in MEA and VOZ ($r_S = 0.618$, p = 0.043); iii) Fungal counts in DG18 and VOZ ($r_S = 0.636$, p = 0.035); iv) Fungal counts in ITZ and POZ ($r_S = 0.733$ p = 0.010), revealing that higher values of bacteria in TSA are related to higher values of fungal counts in MEA and DG18. Higher fungal counts on MEA and DG18 are related to higher values on VOZ. Similar results were obtained between ITZ and POZ (Table 6).

In case of cleaning cloths, statistically significant correlations were detected between: i) bacteria values in TSA and VRBA ($r_S = 0.689$, p =

Table 5
Distribution of IC50 values in FFH samples.

IC50	A549					SK				
	20 mm2/ml	10 mm2/ml	5 mm2/ml	2.5 mm2/ml	1.25 mm2/ml	20 mm2/ml	10 mm2/ml	5 mm2/ml	2.5 mm2/ml	1.25 mm2/ml
Filters (N = 90)	0	0	0	0	0	0	0	0	0	0
EDC (N = 61)	0	6.6%	0	0	0	0	1.6%	1.6%	0	0
Uniform names + Fireman ranks (N = 67)	4.5%	0.0%	1.5%	3.0%	1.5%	0.0%	3.0%	1.5%	0	0
Mops (N = 14)	21.4%	14.3%	21.4%	7.1%	0.0%	21.4%	0	0	0	0
cleaning cloths (N = 58)	8.6%	6.9%	0.0%	1.7%	0.0%	12.1%	0.0%	1.7%	0	0
IC50	**500 mg/ml**	**250 mg/ml**	**125 mg/ml**	**62.5 mg/ml**	**31.25 mg/ml**	**500 mg/ml**	**250 mg/ml**	**125 mg/ml**	**62.5 mg/ml**	**31.25 mg/ml**
Settled dust (N = 11)	9.1%	9.1%	36.4%	27.3%	18.2%	54.5%	18.2%	9.1%	9.1%	0
IC50	**50 μl/ml**	**25 μl/ml**	**12.5 μl/ml**			**50 μl/ml**	**25 μl/ml**	**12.5 μl/ml**		
Air samples (Coriolis device) (N = 99)	7.1%	2.0%	1.0%			3.0%	0	0		

Table 6
Relationship between sampling method (EDC: electrostatic dust collectors) and quantitative results obtained through culture dependent methods for bacteria (VRBA: violet red bile agar) and fungi (MEA: malt extract agar; DG18: dichloran-glycerol agar; ITZ: Itraconazole; VOZ: Voriconazole; POZ: Posaconazole by Spearman's correlation coefficient).

Sampling method	Media	VRBA	MEA	DG18	ITZ	VOZ	POZ
Filters	ITZ	−0.076	0.391	0.427	–	0.755**	0,373
Millipore	TSA	−0.014	0.618*	−0.009	–	–	–
EDC	TSA	0.060	0.633*	0.629*	0.501	0.483	0.457
	MEA	–	–	0.900**	0.573	0.618*	0.314
	DG18	–	–	–	0.564	0.636*	0.442
	ITZ	–	–	–	–	0.936**	0.733*
Cleaning cloths	TSA	0.689*	0.411	0.033	−0.028	0.167	−0.129
	VRBA	–	0.605*	−0.010	0.015	0.039	0.123
Mops	TSA	0.701*	0.233	0.399	−0.174	0.475	−0.434
	VRBA	–	−0.051	−0.039	0.070	0.631*	−0.268
Settled dust	TSA	1.000**	0.647*	0.543	−0.012	−0.168	−0.370
	VRBA	–	0.647*	0.543	−0.012	−0.168	−0.370
Swabs	VRBA	–	0.667*	0.516	–	–	–
Name tags	VRBA	–	−0.302	−0.609*	0.616*	−0.695*	0.424

*Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed).

0.036), same results were obtained for mops ($r_S = 0.701$, $p = 0.016$) ii) bacteria counts in VRBA and fungal counts in MEA ($r_S = 0.605$, $p = 0.049$). Suggesting that a higher bacterial count in TSA is related to a higher bacterial count in VRBA for cleaning cloths and mops. Also, higher bacterial counts in VRBA are related to higher values of fungal counts in MEA for cleaning cloths (Table 6).

In mops used for cleaning, statistically significant correlations were obtained between: bacterial counts in VRBA and fungal counts in VOZ ($r_S = 0.631$, $p = 0.037$), being directly correlated. Similar associations were obtained for settled dust between: i) Bacteria values in TSA and VRBA and fungal values in MEA ($r_S = 0.647$, $p = 0.031$) ($r_S = 0.647$, $p = 0.031$) respectively. Results obtained from swabs reported statistically significant correlations between bacteria values in VRBA and fungal values in MEA ($r_S = 0.667$, $p = 0.016$) (Table 6).

Name tags results showed statistically significant correlations between: i) Bacterial contamination in VRBA and fungal counts in DG18 ($r_S = -0.609$, $p = 0.016$) ITR ($r_S = 0.616$, $p = 0.016$) and VOZ ($r_S = -0.695$, $p = 0.016$) revealing that higher counts of bacteria in VRBA are associated to lower fungal counts in DG18 and VOZ, while higher bacterial counts in VRBA are related to higher fungal counts in ITZ (Table 6).

Regarding cytotoxicity, only significant correlations were detected between A549 cells and fungal counts in DG18 ($r_S = -0.575$, $p = 0.020$) and SK cells ($r_S = 0.622$, $p = 0.041$), revealing that higher values in A549 cells are related with lower fungal counts in DG18 and higher values in SK cells (Supplementary material - Table S5).

4. Discussion

Physical injuries and exposure to smoke and chemical hazards are more often considered as firefighters' occupational exposure. Nevertheless, as workers who interface closely with the natural environment and have contact with contaminated soils, water, animals and infrastructures, firefighters are also likely to be at increased risk of exposure to pathogenic microorganisms [27]. Moreover, exposure to smoke from wildfires may have significant health effects compromising lung function, such as asthma and COPD [28], with growing evidence suggesting COPD patients as being at higher risk of developing invasive pulmonary aspergillosis [29,30]. In the context of climate change and predicted increase of global temperature [31], evidence suggests that firefighters will need enhanced protection to reduce exposures to several risk factors, including pathogens. Thus, occupational health research agenda needs to be set and a better characterization of the firefighters occupational related health hazards is required to gather broader data to support the implementation of new or improved surveillance programs.

Until now, data covering a wider spectrum of the microbial contamination, obtained by a multi-approach on sampling methods and assays in FFH have not been reported, and this omission has probably barred the implementation of suitable preventive measures. This study comprises a comprehensive sampling and analyses protocol to achieve the most accurate exposure scenario in what concerns the FFH environment. In fact, besides the microbial contamination characterization applying culture based-methods, critical for the azole screening analyses, were applied molecular tools to detect the pandemic virus and the indicators of harmful fungal presence [32,33]. In addition, mycotoxins detection allowed the evaluation of contaminants that are not usually evaluated in occupational environments but, due to the health effects related to several mycotoxins, keeping exposure as low as possible in the workplaces should undoubtedly be an objective to follow [34,35]. It was also possible to ponder about the possible health effects due to exposure through the cytotoxicity evaluation performed.

In what concerns microbial contamination, surface swabs were the matrix with the highest values of total bacteria and fungi, followed by filters. The higher counts associated to passive sampling methods were expected when compared to the results from active sampling methods, since passive sampling enables the characterization of contamination from a longer period of time [36].

The Portuguese legislation for Indoor air quality (IAQ) suggest that fungal load ratio between indoor and outdoor should be lower than 1 (I/O < 1). Concerning bacteria, indoors' load should not exceed the outdoor by 350 colony-forming units (CFU).m^{-3} (Indoor+350 UFC.$m.^{-3}$< outdoor) [33]. When applying this quantitative criteria (ratio I/O < 1), most of the FFH were above the stipulated limit for bacteria load (63.63%) and fungal load (45.45%) suggesting a critical environment regarding the microbiological contamination [33,37,38].

Regarding fungal diversity, the matrix presenting higher number of different fungal species were the EDC (18 MEA; 15 DG18), while for *Aspergillus* genera were the air samples obtained by Andersen device (5 sections MEA; 7 sections DG18). In fact, A*spergillus* genera was identified in air and samples from passive sampling methods, as previously reported in different indoor environments [37,39–41]. The diversity of *Aspergillus* sections in all the six-stages from Andersen device might constitute a health hazard [37], since it represents the human respiratory system and microbial penetrability in the lung (Viegas et al., 2021e), thus suggesting fungal exposure through inhalation and highlighting a possible risk factor for respiratory diseases [37]. As a matter of fact, the detection of sections *Candidi* and *Nidulantes* should be preconized due to its toxigenic potential and clinical relevance [33,42]. In addition, the different results regarding the diversity assessment performed among sampling areas, comparing results obtained by an active (Anderson) and a passive sampling method (EDC) corroborates the importance to apply a wide range of sampling methods when performing

microbiologic agents occupational exposure assessments [2,5,37]. All the sampling areas, presenting higher diversity, in both sampling methods may have different reasons for the obtained results. In fact, there is a wide range of contamination sources that can potentiate fungal contamination indoors, such as human occupancy and their activities, humidity levels, ventilation, environmental characteristics, water infiltrations, building and decoration materials and outdoor air, since the ventilation was provided through the open of windows and doors (WHO 2009; [33]. However, common reasons can prevail among all the FFH, such as damp, leakages and cracks on the walls observed during the sampling campaign [43].

Results from passive sampling methods evidence *Cladosporium* as the most frequent genera, in accordance with previous results [40], followed by *Penicillium* sp. in MEA and DG18. *Aspergillus* sections (*Circumdati, Nigri* and *Fumigati)* were also recurrent among matrices from passive sampling methods, being identified as mycotoxins producers [42]. Also, the prevalence of *Aspergillus* sp. in mops and cloths used for common cleaning of FFH might constitute a potential source of cross-contamination [15]. Regular cleaning of the surfaces with suitable cleaning agents and dedicated material (e.g specific mobs for each area/surface) will contribute to prevent the contamination found and avoid cross contamination. Additionally, avoid bringing and keeping indoors food and other commodities that are prone to fungal contamination should also be consider as preventive measure.

The highest prevalence of *Aspergillus* sp. was obtained in DG18. Such result might be related to the restrictive character of the media, limiting the development of fast-growing fungal species. Thus, both media (MEA and DG18) should be used in order to obtain a more comprehensive fungal characterization [12,44,45].

Regarding fungal resistance profile, *Cladosporium* sp., *Penicillium* sp. and *Aspergillus* sp. grow in more than one azole supplemented media evidence the trend of multidrug resistance [37]. AMB MICs >2 μg/ml and ITZ MICs >8 μg/ml are associated with treatment failure and clinical resistance to these agents. No isolate belonging to *Fumigati* had ITZ MICs ≥8 μg/ml, nor AMB MICs >2 μg/ml, suggesting that these isolates are susceptible to the tested azoles. This was in accordance with Sabino and colleagues' study [46], and in contrast to what was reported by other authors [47–51].

If we consider the MIC value of >2 μg/ml to AMB, ITZ, or VCZ, or >0.25 μg/ml for PSZ as a cutoff for resistance, as described by Alastruey-Isquerdo et al. (2014), 36% (5/14) *Fumigati* isolates would now be considered resistant. Noteworthy, all of them were collected by passive sampling, most (4/5) by EDC.

Regarding azole drugs, 3/14 isolates showed MICs ≥2 μg/ml to ITZ and 1/14 isolates to VCZ, and 4/14 isolates showed MICs ≥0.25 μg/ml to PSZ. VCZ seems to be the most effective antifungal in vitro, with only one isolate having MICs ≥2 μg/ml.

These data raise awareness and concern about the issue of antifungal resistance in the environment, and to the eventual emergence of resistant strains in occupational exposure scenarios such as FFH. A significant increase in azole resistance, specifically for *Aspergillus* section *Fumigati*, and high frequencies of resistant isolates have already been described in Portugal [46,52] and in other European countries [24,53,54].

Culture-based methods allowed us to identify *Aspergillus* section *Fumigati*, in a very short number of samples (18 out of 379), whereas molecular tools detected this section in a larger number of samples (146 out of 379). *Aspergillus* sections *Nidulantes* and *Circumdati* were only detected through molecular tools in one sample and were not identified through culture-based methods. However, section *Fumigati* was identified in 10 samples and not detected through qPCR. Molecular technologies enable microbe identification that is exact, rapid, specific, and sensitive. They can also distinguish toxigenic strains from ordinary strains within specific fungal species and detect dead or dormant microorganisms [42,55]. Culture-based methods, on the other hand, are of upmost importance, because the viability of bioburden is crucial for estimating health risks, since it impacts on biological processes such as inflammatory and cytotoxic reactions [42,56]. This highlights the concept of combining molecular and culture-based approaches in exposure assessment studies [22]. In addition, and without intention of weakening the advantages of molecular analysis, in exposure assessment studies, where the exposure in mostly occurring by inhalation and the outcomes of respiratory diseases may vary considerably with fungal viability is of critical importance to apply culture for the fungal assessment or isolates recovery. This corroborates the importance to perform the identification by culture, using the same procedure as in clinical samples, since it is the gold standard for diagnosis of fungal infections and allow susceptibility testing [2,57–59]. Overall, these results corroborate that culture based-methods and molecular tools are complementary and should be used side-by-side whenever possible, as it was reported in previous studies performed in different occupational environments [5,37,45,60].

Despite the sampling volume from the impingement and the detection technique were the ones widely recommended [42,61,62], all the results were negative for SARS-CoV-2. However, some studies [63,64] have applied the recently developed digital PCR technique, which has higher sensitivity and precision when compared to standard qPCR, allowing the detection of viral nucleic acid present at low concentrations [62]. Still, the obtained results suggest the efficacy of the present measures in place. In fact, disinfection products and procedures were focusing in the elimination of the pandemic agent and, probably, neglecting other measures to avoid microorganisms, besides SARS-CoV-2, to thrive. In addition, the negative results can be due the positive impact of vaccination in Portugal since, at the time, the firefighters were the workers population already vaccinated. The same results were found in previous study performed in higher education institutes from Portugal where the vaccination rate was lower than in FFH [42].

Previous reports [34,35] have already highlighted the importance of documenting mycotoxins exposure in workplaces since fungi are not suitable indicators for mycotoxins presence [65]. This is mainly due to the fact that mycotoxins can be present in the environment long after fungi have been eliminated and not all the fungi produce mycotoxins [66,67]. Additionally, and has already described in other occupational environments [34,37], exposure to mycotoxins is frequently characterized by simultaneous exposure to several mycotoxins bringing new challenges to the risk assessment associated to this exposure due to the possible mycotoxins interaction. This aspect is due to several factors, including the ability of some fungi to produce several mycotoxins [34,35,68]. Also in the FFH we found several mycotoxins (fumonisin B2, nivalenol, mycophenolic acid and sterigmatocystin) and, also similar to other occupational environments, exposure might be occurring by inhalation [34,35]. The preventive measures to adopt to avoid mycotoxins contamination of the workplace environment are the same to prevent fungi presence, such as cleaning frequently the surfaces and avoid bringing and keeping indoors food and other commodities that might be prone to fungal contamination.

The fact that lower fungal burden (DG18) was correlated with lower cytotoxicity in lung epithelial cells, also correlated with renal cells, seems to imply that the DG18 mycobiota might be responsible for some of the observed cytotoxic effect in FFH samples. Interestingly, DG18 was the media that enabled the identification of the highest prevalence of *Aspergillus* sp. in FFH samples. This fungal genus, which includes some mycotoxigenic species, might have a role in the imparted cytotoxicity, along with other contaminants that might be present in the collected samples, such as metals, polycyclic aromatic hydrocarbons, bisphenols, phthalates and brominated flame retardants [16], particularly in lung cells. While firefighter's exposure to certain toxic agents, especially combustion products, during fires is well known, this study suggests that exposures in FFH also contribute to their augmented cancer risk and lesion in the airways [16].

In future studies the contribution of *Aspergillus* sections isolates to the

overall cytotoxicity should be assessed and the mutations from section *Fumigati* isolates target [52,69,70]. In those studies a refine genetic characterization from isolates will be performed as in previous assessments held in different occupational environments [24,52].

5. Conclusions

The multi-approach on sampling methods (active and passive) and laboratory assays (culture based-methods, molecular tools, mycotoxins detection, cytotoxicity evaluation) improved data findings, enabling a more detailed and accurate risk characterization. Overall, it was raised concern regarding the following findings: a) microbial contamination didn't comply with Portuguese IAQ legal requirements; b) fungal exposure through inhalation underlining a possible risk factor for respiratory diseases; c) prevalence of *Aspergillus* sp. in cleaning materials that might represent a potential source of cross-contamination; d) widespread of *Aspergillus* section *Fumigati* in all the FFH analysed; e) trend of multidrug resistance found among fungi with focus on *Fumigati* isolates; f) detection of several mycotoxins and; g) potential role of *Aspergillus* genus in the cytotoxicity found, particularly in lung cells.

CRediT authorship contribution statement

Carla Viegas: Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis. **Bianca Gomes:** Formal analysis, Writing – original draft. **Raquel Pimenta:** Writing – original draft, Formal analysis. **Marta Dias:** Formal analysis, Writing – original draft. **Renata Cervantes:** Writing – original draft, Formal analysis. **Liliana Aranha Caetano:** Methodology, Writing – original draft, Writing – review & editing. **Elisabete Carolino:** Writing – original draft, Formal analysis. **Magdalena Twarużek:** Formal analysis, Funding acquisition, Writing – original draft. **Ewelina Soszczyńska:** Formal analysis. **Robert Kosicki:** Formal analysis. **Susana Viegas:** Writing – review & editing, Writing – original draft, Formal analysis.

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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References

[1] K.T. Rim, C.H. Lim, Biologically hazardous agents at work and efforts to protect workers' health: a review of recent reports, Saf. Health Work 5 (2014) 43–52, https://doi.org/10.1016/j.shaw.2014.03.006.

[2] C. Viegas, B. Gomes, M. Dias, E. Carolino, L.A. Caetano, Occupational exposure to Aspergillus section Fumigati in firefighter headquarters, Microorganisms 9 (2021), https://doi.org/10.3390/microorganisms9102112.

[3] G. Jacobsen, I. Schaumburg, T. Sigsgaard, V. Schlünssen, Non-malignant respiratory diseases and occupational exposure to wood dust. Part I. Fresh wood and mixed wood industry, Ann. Agric. Environ. Med. 17 (2010) 15–28.

[4] A.M. Madsen, M. Raulf, P. Duquenne, P. Graff, M. Cyprowski, A. Beswick, S. Laitinen, P.U. Rasmussen, M. Hinker, A. Kolk, R.L. Górny, A. Oppliger, B. Crook, Review of biological risks associated with the collection of municipal wastes, Sci. Total Environ. 791 (2021), https://doi.org/10.1016/j.scitotenv.2021.148287.

[5] C. Viegas, T. Faria, L.A. Caetano, E. Carolino, A.Q. Gomes, S. Viegas, Aspergillus spp. prevalence in different Portuguese occupational environments: what is the real scenario in high load settings? J. Occup. Environ. Hyg. 14 (2017) 771–785, https://doi.org/10.1080/15459624.2017.1334901.

[6] C. Viegas, L.A. Caetano, J. Cox, M. Korkalainen, S.R. Haines, K.C. Dannemiller, S. Viegas, T. Reponen, The effects of waste sorting in environmental microbiome, THP-1 cell viability and inflammatory responses, Environ. Res. 185 (2020), https://doi.org/10.1016/j.envres.2020.109450.

[7] C. Viegas, M. Twarużek, M. Dias, B. Almeida, E. Carolino, E. Soszczyńska, S. Viegas, L. Aranha Caetano, Cytotoxicity of filtering respiratory protective devices from the waste sorting industry: a comparative study between interior layer and exhalation valve, Environ. Int. 155 (2021), https://doi.org/10.1016/j.envint.2021.106603.

[8] J.E. Smith, J.G. Anderson, C.W. Lewis, Y.M. Murad, Cytotoxic fungal spores in the indoor atmosphere of the damp domestic environment, FEMS Microbiol. Lett. 100 (1992) 337–343, https://doi.org/10.1111/j.1574-6968.1992.tb14061.x.

[9] M. Hanelt, M. Gareis, B. Kollarczik, Cytotoxicity of mycotoxins evaluated by the MTT-cell culture assay, Mycopathologia 128 (1994) 167–174, https://doi.org/10.1007/BF01138479.

[10] E. Maličev, H. Chowdhury, P. Maček, K. Sepčić, Effect of ostreolysin, an Asp-hemolysin isoform, on human chondrocytes and osteoblasts, and possible role of Asp-hemolysin in pathogenesis, Med. Mycol. 45 (2007) 123–130, https://doi.org/10.1080/13693780601039615.

[11] E. Piecková, Adverse health effects of indoor moulds, Arh. Hig. Rada. Toksikol. 63 (2012) 545–549, https://doi.org/10.2478/10004-1254-63-2012-2221.

[12] S. Viegas, L.A. Caetano, M. Korkalainen, T. Faria, C. Pacífico, E. Carolino, A. Q. Gomes, C. Viegas, Cytotoxic and inflammatory potential of air samples from occupational settings with exposure to organic dust, Toxics 5 (2017), https://doi.org/10.3390/toxics5010008.

[13] C. Ladeira, G. Gajski, M. Meneses, M. Gerić, S. Viegas, The genotoxicity of an organic solvent mixture: a human biomonitoring study and translation of a real-scenario exposure to in vitro, Regul. Toxicol. Pharmacol. 116 (2020), https://doi.org/10.1016/j.yrtph.2020.104726.

[14] M.Z. Jeddi, N.B. Hopf, S. Viegas, R. Pasanen-Kase, Use of effect Biomarkers for regulatory risk assessment of chemical mixtures, Biomarkers J 7 (2021).

[15] C. Viegas, P. Sousa, M. Dias, L.A. Caetano, E. Ribeiro, E. Carolino, M. Twarużek, R. Kosicki, S. Viegas, Bioburden contamination and Staphylococcus aureus colonization associated with firefighter's ambulances, Environ. Res. 197 (2021), https://doi.org/10.1016/j.envres.2021.111125.

[16] E.H. Sparer, D.P. Prendergast, J.N. Apell, M.R. Bartzak, G.R. Wagner, G. Adamkiewicz, J.E. Hart, G. Sorensen, Assessment of ambient exposures firefighters encounter while at the fire station: an exploratory study, J. Occup. Environ. Med. 59 (2017) 1017–1023, https://doi.org/10.1097/JOM.0000000000001114.

[17] Y. Nigam, J. Cutter, A preliminary investigation into bacterial contamination of Welsh emergency ambulances, Emerg. Med. J. 20 (2003) 479–482, https://doi.org/10.1136/emj.20.5.479.

[18] J.D. Sexton, K.A. Reynolds, Exposure of emergency medical responders to methicillin-resistant Staphylococcus aureus, Am. J. Infect. Control 38 (2010) 368–373, https://doi.org/10.1016/j.ajic.2010.01.004.

[19] M.C. Roberts, B. David, Environment surface sampling in 33 Washington State fire stations for methicillin-resistant and methicillin-susceptible Staphylococcus aureus, Am. J. Infect. Control 42 (2014) 591–596, https://doi.org/10.1016/j.ajic.2014.02.019.

[20] D. Farcas, F.M. Blachere, M.L. Kashon, D. Sbarra, D. Schwegler-Berry, J.O. Stull, J. D. Noti, Survival of Staphylococcus aureus on the outer shell of fire fighter turnout gear after sanitation in a commercial washer/extractor, J. Occup. Med. Toxicol. 14 (2019) 1–14, https://doi.org/10.1186/s12995-019-0230-4.

[21] C. McGuire-Wolfe, Firefighters fighting Germs - evaluation of a disinfecting protocol for use in the fire service, Infect. Control Hosp. Epidemiol. 41 (2020) s519–s520, https://doi.org/10.1017/ice.2020.1202.

[22] C. Viegas, L.A. Caetano, S. Viegas, Occupational exposure to Aspergillus section Fumigati: tackling the knowledge gap in Portugal, Environ. Res. 194 (2021), https://doi.org/10.1016/j.envres.2020.110674.

[23] G. De Hoog, J. Guarro, J. Gebé, M. Figueras, Atlas of Clinical Fungi, second ed., Centraalbureau voor Schimmelcultures, Utrecht (The Netherlands), 2000.

[24] C. Viegas, B. Almeida, L. Aranha Caetano, A. Afanou, A. Straumfors, C. Veríssimo, P. Gonçalves, R. Sabino, Algorithm to assess the presence of Aspergillus fumigatus resistant strains: the case of Norwegian sawmills, Int. J. Environ. Health Res. (2020), https://doi.org/10.1080/09603123.2020.1810210.

[25] United States Environmental Protection Agency, EPA Observational Economy Series, 1, 1995. Composite Sampling 1.

[26] S. Prinsloo, R. Pieters, C.C. Bezuidenhout, A cell viability assay to determine the cytotoxic effects of water contaminated by microbes, South Afr. J. Sci. 109 (2013), https://doi.org/10.1590/sajs.2013/20120069.

[27] S. Altizer, R.S. Ostfeld, P.T.J. Johnson, S. Kutz, C.D. Harvell, Climate change and infectious diseases: from evidence to a predictive framework, Science 341 (6145) (2013) 514–519.

[28] J.C. Slaughter, J.Q. Koenig, T.E. Reinhardt, Association between lung function and exposure to smoke among firefighters at prescribed burns, J. Occup. Environ. Hyg. 1 (1) (2004) 45–49.
[29] P. Bulpa, A. Dive, Y. Sibille, Invasive pulmonary aspergillosis in patients with chronic obstructive pulmonary disease, Eur. Respir. J. 30 (2007) 782–800.
[30] M. Kousha, R. Tadi, Soubani A.O. Pulmonary aspergillosis: a clinical review, Eur. Respir. Rev. 20 (2011) 156–174.
[31] R. Assunção, C. Martins, S. Viegas, C. Viegas, L.S. Jakobsen, S. Pires, P. Alvito, Climate change and the health impact of aflatoxins exposure in Portugal - an overview, Food Addit. Contam. Part A Chem Anal Control Expo Risk Assess 35 (8) (2018) 1610–1621.
[32] ACGIH, Guidelines for the assessment of bioaerosols in the indoor environment, Am. Conf. Gov. Ind. Hyg. (1989).
[33] Agência Portuguesa do Ambiente, Qualidade Do Ar Em Espaços Interiores Um Guia Técnico 2010, 2010.
[34] S. Viegas, C. Viegas, A. Oppliger, Occupational exposure to mycotoxins: current knowledge and prospects, Ann. Work Expo. Heal. 62 (2018) 923–941, https://doi.org/10.1093/annweh/wxy070.
[35] S. Viegas, C. Viegas, C. Martins, R. Assunção, Occupational exposure to mycotoxins - different sampling strategies telling a common story regarding occupational studies performed in Portugal (2012-2020), Toxins 12 (2020), https://doi.org/10.3390/toxins12080513.
[36] C. Viegas, M. Dias, B. Almeida, E. Vicente, C. Candeias, L. Aranha Caetano, E. Carolino, C. Alves, Loading rates of dust and bioburden in dwellings in an Inland city of Southern Europe, Atmosphere 12 (2021), https://doi.org/10.3390/atmos12030378.
[37] C. Viegas, B. Almeida, A. Monteiro, L.A. Caetano, E. Carolino, A.Q. Gomes, M. Twarużek, R. Kosicki, G. Marchand, S. Viegas, Bioburden in health care centers: is the compliance with Portuguese legislation enough to prevent and control infection? Build. Environ. 160 (2019) https://doi.org/10.1016/j.buildenv.2019.106226.
[38] J. Cox, H. Mbareche, W.G. Lindsley, C. Duchaine, Field sampling of indoor bioaerosols, Aerosol Sci. Technol. 54 (2020) 572–584, https://doi.org/10.1080/02786826.2019.1688759.
[39] C. Alberti, A. Bouakline, P. Ribaud, C. Lacroix, P. Rousselot, T. Leblanc, F. Derouin, Relationship between environmental fungal contamination and the incidence of invasive aspergillosis in haematology patients, J. Hosp. Infect. 48 (2001) 198–206, https://doi.org/10.1053/jhin.2001.0998.
[40] C.A. Ramos, C. Viegas, S.C. Verde, H.T. Wolterbeek, S.M. Almeida, Characterizing the fungal and bacterial microflora and concentrations in fitness centres, Indoor Built Environ. 25 (2016) 872–882, https://doi.org/10.1177/1420326X15587954.
[41] C. Viegas, M. Twaruzek, R. Lourenço, M. Dias, B. Almeida, L.A. Caetano, E. Carolino, A.Q. Gomes, R. Kosicki, E. Soszczyńska, S. Viegas, Bioburden assessment by passive methods on a clinical pathology service in one central hospital from Lisbon: what can it tell us regarding patients and staff exposure? Atmosphere 11 (2020) https://doi.org/10.3390/atmos11040351.
[42] C. Viegas, R. Pimenta, M. Dias, B. Gomes, M. Brito, L. Aranha Caetano, E. Carolino, A.Q. Gomes, Microbiological contamination assessment in higher education institutes, Atmosphere 12 (2021), https://doi.org/10.3390/atmos12081079.
[43] A. Rajasekar, R. Balasubramanian, Assessment of airborne bacteria and fungi in food courts, Build. Environ. 46 (2011) 2081–2087.
[44] C. Viegas, B. Almeida, A.Q. Gomes, E. Carolino, L.A. Caetano, Aspergillus spp. prevalence in Primary Health Care Centres: assessment by a novel multi-approach sampling protocol, Environ. Res. 175 (2019) 133–141, https://doi.org/10.1016/j.envres.2019.05.015.
[45] C. Viegas, G.T.A. Fleming, A. Kadir, B. Almeida, L.A. Caetano, A.Q. Gomes, M. Twarużek, R. Kosicki, S. Viegas, A.M. Coggins, Occupational exposures to organic dust in Irish bakeries and a pizzeria restaurant, Microorganisms 8 (2020), https://doi.org/10.3390/microorganisms8010118.
[46] R. Sabino, E. Carolino, C. Veríssimo, M. Martinez, K.V. Clemons, D.A. Stevens, Antifungal susceptibility of 175 Aspergillus isolates from various clinical and environmental sources, Med. Mycol. 54 (2016) 740–756, https://doi.org/10.1093/mmy/myw024.
[47] J. Chen, H. Li, R. Li, D. Bu, Z. Wan, Mutations in the cyp51A gene and susceptibility to itraconazole in Aspergillus fumigatus serially isolated from a patient with lung aspergilloma, J. Antimicrob. Chemother. 55 (2005) 31–37, https://doi.org/10.1093/jac/dkh507.
[48] T.M. Diaz-Guerra, E. Mellado, M. Cuenca-Estrella, J.L. Rodriguez-Tudela, A point mutation in the 14α-sterol demethylase gene cyp51a contributes to itraconazole resistance in Aspergillus fumigatus, Antimicrob. Agents Chemother. 47 (2003) 1120–1224, https://doi.org/10.1128/AAC.47.3.1120-1124.2003.
[49] S.J. Howard, I. Webster, C.B. Moore, R.E. Gardiner, S. Park, D.S. Perlin, D. W. Denning, Multi-azole resistance in Aspergillus fumigatus, Int. J. Antimicrob. Agents 28 (2006) 450–453, https://doi.org/10.1016/j.ijantimicag.2006.08.017.
[50] P.A. Mann, R.M. Parmegiani, S.Q. Wei, C.A. Mendrick, X. Li, D. Loebenberg, B. DiDomenico, R.S. Hare, S.S. Walker, P.M. McNicholas, Mutations in Aspergillus fumigatus resulting in reduced susceptibility to posaconazole appear to be restricted to a single amino acid in the cytochrome P450 14α-demethylase, Antimicrob. Agents Chemother. 47 (2003) 577–581, https://doi.org/10.1128/AAC.47.2.577-581.2003.
[51] A.M. Nascimento, G.H. Goldman, S. Park, S.A.E. Marras, G. Delmas, U. Oza, K. Lolans, M.N. Dudley, P.A. Mann, D.S. Perlin, Multiple resistance mechanisms among Aspergillus fumigatus mutants with high-level resistance to itraconazole, Antimicrob. Agents Chemother. 47 (2003) 1719–1726, https://doi.org/10.1128/AAC.47.5.1719-1726.2003.
[52] P. Gonçalves, A. Melo, M. Dias, B. Almeida, L.A. Caetano, C. Veríssimo, C. Viegas, R. Sabino, Azole-resistant aspergillus fumigatus harboring the tr34/l98h mutation: first report in Portugal in environmental samples, Microorganisms 9 (2021) 1–10, https://doi.org/10.3390/microorganisms9010057.
[53] S.J. Howard, D. Cerar, M.J. Anderson, A. Albarrag, M.C. Fisher, A.C. Pasqualotto, M. Laverdiere, M.C. Arendrup, D.S. Perlin, D.W. Denning, Frequency and evolution of azole resistance in Aspergillus fumigatus associated with treatment failure, Emerg. Infect. Dis. 15 (2009) 1068–1076, https://doi.org/10.3201/eid1507.090043.
[54] P.E. Verweij, E. Snelders, G.H. Kema, E. Mellado, W.J. Melchers, Azole resistance in Aspergillus fumigatus: a side-effect of environmental fungicide use? Lancet Infect. Dis. 9 (2009) 789–795, https://doi.org/10.1016/S1473-3099(09)70265-8.
[55] L. MacNeil, T. Kauri, W. Robertson, Molecular techniques and their potential application in monitoring the microbiological quality of indoor air, Can. J. Microbiol. 41 (1995) 657–665, https://doi.org/10.1139/m95-091.
[56] A.M. Madsen, M.W. Frederiksen, M.H. Jacobsen, K. Tendal, Towards a risk evaluation of workers' exposure to handborne and airborne microbial species as exemplified with waste collection workers, Environ. Res. 183 (2020), https://doi.org/10.1016/j.envres.2020.109177.
[57] T.R. Kozel, B. Wickes, Fungal diagnostics, Cold Spring Harb. Perspect. Med. 4 (4) (2014) a019299, https://doi.org/10.1101/cshperspect.a019299.
[58] K. Yoo, T.K. Lee, E.J. Choi, J. Yang, S.K. Shukla, S. Hwang, J. Park, Molecular approaches for the detection and monitoring of microbial communities in bioaerosols: a review, J. Environ. Sci. 51 (2016) 234–247.
[59] Clinical Laboratory Standards Institute (CLSI). https://clsi.org/about/blog/detection-and-culture-of-fungi-in-clinical-specimens/, 2022, 12 of January 2022.
[60] C. Viegas, T. Faria, M. dos Santos, E. Carolino, A.Q. Gomes, R. Sabino, S. Viegas, Fungal burden in waste industry: an occupational risk to be solved, Environ. Monit. Assess. (2015), https://doi.org/10.1007/s10661-015-4412-y.
[61] Bertin Instruments, Air monitoring—coriolis air samplers collect biological Particles in the air which offer new Perspectives for the control of airborne contamination Thanks to its liquid sample, Available online: https://www.bertininstruments.com/products-range/air-samplers/, 2020. September 2021.
[62] P. Pena, J. Morais, A. Quintal Gomes, C. Viegas, Sampling methods and assays applied in SARS-CoV-2 exposure assessment, Sci. Total Environ. 775 (2021) 145903, https://doi.org/10.1016/j.scitotenv.2021.145903.
[63] R. Gonzalez, K. Curtis, A. Bivins, K. Bibby, M.H. Weir, K. Yetka, H. Thompson, D. Keeling, J. Mitchell, D. Gonzalez, COVID-19 surveillance in Southeastern Virginia using wastewater-based epidemiology, Water Res. 186 (2020) 116296, https://doi.org/10.1016/j.watres.2020.116296.
[64] Y. Liu, Z. Ning, Y. Chen, M. Guo, Y. Liu, N.K. Gali, L. Sun, Y. Duan, J. Cai, D. Westerdahl, X. Liu, K. Xu, K.-F. Ho, H. Kan, Q. Fu, K. Lan, Aerodynamic analysis of SARSCoV-2 in two Wuhan hospitals, Nature 582 (7813) (2020) 557–560, https://doi.org/10.1038/s41586-020-2271-3.
[65] A.S. Halstensen, K.C. Nordby, W. Eduard, S.S. Klemsdal, Real-time PCR detection of toxigenic Fusarium in airborne and settled grain dust and associations with trichothecene mycotoxins, J. Environ. Monit. 8 (2006) 1235–1241, https://doi.org/10.1039/b609840a.
[66] A.S. Halstensen, Species-specific fungal DNA in airborne dust as surrogate for occupational mycotoxin exposure? Int. J. Mol. Sci. 9 (2008) 2543–2558, https://doi.org/10.3390/ijms9122543.
[67] L. Alborch, M.R. Bragulat, M.L. Abarca, F.J. Cabañes, Effect of water activity, temperature and incubation time on growth and ochratoxin A production by Aspergillus Niger and Aspergillus carbonarius on maize kernels, Int. J. Food Microbiol. 147 (2011) 53–57, https://doi.org/10.1016/j.ijfoodmicro.2011.03.005.
[68] S. Wallin, L. Gambacorta, N. Kotova, E. Warensjö Lemming, C. Nälsén, M. Solfrizzo, M. Olsen, Biomonitoring of concurrent mycotoxin exposure among adults in Sweden through urinary multi-biomarker analysis, Food Chem. Toxicol. 83 (2015) 133–139, https://doi.org/10.1016/j.fct.2015.05.023.
[69] C. Viegas, M. Twarużek, B. Almeida, M. Dias, E. Ribeiro, E. Carolino, E. Soszczyńska, L.A. Caetano, Cytotoxicity of Aspergillus section Fumigati isolated from health care environments, J. Fungi 7 (2021) 839, https://doi.org/10.3390/jof7100839.
[70] C. Viegas, M. Twarużek, M. Dias, E. Carolino, E. Soszczyńska, L. Aranha Caetano, Cytotoxicity of Aspergillus section Fumigati isolates recovered from protection devices used on waste sorting industry, Toxins 14 (2022) 70, https://doi.org/10.3390/toxins14020070.