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Development of an Indexed Score to Identify the Most Suitable Sampling Method to Assess Occupational Exposure to Fungi

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Abstract: The sampling approach is of utmost importance to obtain data regarding microbial viability and truly assess workers' potential health effects. The purpose of this assessment is to create a score which will provide up-to-date information to identify the most suitable sampling method to assess occupational exposure to fungi. Data from a sampling campaign performed at Firefighters Headquarters (FFH) was analysed and a score was calculated from one (1) to three (3) for five (5) distinct sample parameters: (a) accuracy; (b) complexity of the field work; (c) cost; (d) complexity in laboratory work; and (e) time taken since the fieldwork until obtaining the fungal contamination characterization. The statistical analysis allowed us to conclude that settled dust and Andersen six-stage were the best sampling methods to perform the assessment of the occupational exposure to fungi at FFH, when considering the number of species. As for the final score, the results showed that surface swabs were the best sampling method. The results obtained for surface swabs highlights the low complexity of this processing combined with the fact that it is a low-cost sampling method. This study reinforces the need to use a wide array of sampling methods when assessing occupational exposure to fungal contamination to ensure an accurate risk characterization.

Keywords: occupational exposure to fungi; sampling methods; indexed score; accuracy; surface swabs

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1. Introduction

The Sustainable Development Goals proposed by WHO, and, more precisely, the goals 3 and 8, highlights the need to guarantee healthy lives and encourage wellbeing and decent work for all, correspondingly [1]. Lately, WHO recognized as priority settings for action the workplaces reporting more than 1.2 million deaths annually due to occupational risks, and the fact that only a small fraction of the general workforce has access to occupational health surveillance [2]. Thus, there is still a need to develop actions aiming to improve workplace safety conditions, and the first steps are exposure assessment and risk characterization in the different settings [3,4].

One of the most important tasks for an industrial hygienist when deciding on the sampling strategy to apply in occupational exposure assessments is to select the most suitable sampling approach, since field work can undermine the results obtained in the laboratory [5]. Regarding microbial contamination assessment, and, more specifically, fungal contamination, the sampling approach is of utmost importance to obtain data regarding microbial viability and truly assess workers´ potential health effects [2,6]. In fact,

the use of culture-dependent methods is vital to predict potential health risks since microbial viability affects inflammatory and cytotoxic responses [1,2]. Additionally, it requires obtaining fungal isolates to characterize the resistance azole profile from a specific occupational environment [5,7,8].

A wide range of sampling methods to assess occupational microbial exposure should be used to try to overcome the limitations of each method and obtain an accurate exposure assessment allowing risk characterization and management [5]. However, after obtaining the results we should analyze the accuracy of each sampling method to decide about future sampling campaigns in the same occupational environment.

Microbial determination procedures can be accomplished by diverse techniques. Regarding sampling methods for bioaerosols assessment, air sampling is recurrent [8], and the impaction method is the most frequently used [9,10]. This sampling approach relies exclusively on culture-based methods [8], enabling microorganisms' quantification and identification [11–13]. However, since it can only evaluate culturable microorganisms, the microbial counts may be undervalued due to the short sampling time or the conditions set on the sampling device, since high velocity of the air flow may result in cell damage, compromising the microorganisms' viability [12,13]. Despite the limitations, active sampling methods are known to be valuable in the characterization of occupational exposure to fungi [14–16]. Passive sampling methods, such as surface swabs [10], electrostatic dust collectors [17,18], and settled dust collection [19,20], can also be applied in the exposure assessment. Indeed, such low-priced techniques are assumed to be more representative of long-term exposure [21]. Thus, to achieve a reliable microbial characterization, sampling campaigns dedicated to microbial exposure assessment should include the use of both active and passive sampling methods [14,19,22].

Several advantages of using scoring rubrics in performance assessments have been advocated, including enhanced scoring uniformity, the ability to allow correct evaluation of difficult abilities, and learning promotion [23]. The major reason for this is because this will allow making expectations and criteria plain, and making feedback and self-assessment easier [6]. The purpose of this assessment was to create a score which will provide up-to-date information to identify the most suitable sampling method to assess occupational exposure to fungi by analyzing different sampling methods. This was achieved considering all the sampling methods characteristics, such as: quantification, accuracy, materials and equipment needed, cost, the need for highly specialized and trained exposure assessors/microbiologists, the complexity for extraction and detection protocols, and the time needed to obtain results from the field to the lab. This assessment was done for the sampling methods applied in an enlarged project focusing on microbial contamination assessment at Firefighters Headquarters (FFH) [4]. As a comparison for the accuracy parameter, statistical studies were conducted, which allowed us to appropriately analyze and compare the score findings. By using five predictor variables, a simple scheme of assessment for good predictive accuracy will be constructed and it can function as a tool to apply in future decision-making processes in different occupational environments.

2. Materials and Methods

2.1. Sampling Approach and Analyses Performed

Eleven firefighter headquarters in the Lisbon district were selected as part of a funded research study to characterize the occupational exposure of firefighters to microbial contamination. The most common activities performed by the firefighters included civil protection and assistance in situations of fire, floods, and landslides, accidents and catastrophes, as well as rescue of pets and wild animals. About 800 firefighters in the headquarters being assessed were usually engaged in these activities. Common areas to all headquarters were assessed, namely: locker rooms, dormitory, canteen, bar, kitchen, living room, reception, administrative room, and gym. The sampling campaign was conducted on one day period during normal working days [4,5]. In most FFH the ventilation

was only ensured through open doors and windows. Information regarding each FFH was obtained through a walk-through survey list used to gather information about all the characteristics that can influence microbial growth and dissemination [4,5].

Regarding the sampling campaign applied at the FFH, a multi-approach protocol was followed covering active and passive sampling methods [24]. A total of 760 indoor air samples were collected by the impaction method using the Andersen six-stage (360 samples) and Millipore air sampler (400 samples) methods, according to the manufacturers' guidelines. Samples were collected onto each plate (190 plates from each culture medium). Two culture media were used for the impaction method, namely: malt extract agar (MEA) supplemented with chloramphenicol (0.05%) and dichloran-glycerol agar (DG18) for fungal assessment.

For the passive methods, floor-surface swabs were performed using a square stencil, and disinfected with 70% alcohol in each sampling (90 samples). Electrostatic dust collectors (EDC) were placed in each FFH sampled area (82 samples). A vacuum cleaner equipped with a filter was used for dust collection of each FFH (11 composite samples) (Table 1). Further information regarding the sampling campaign was previously reported [4].

Table 1. Sampling methods applied in the assessed FFH [4].

Sampling Methods	Description	
Andersen six-stage	This sampling method requires a small, yet specific, and technical, number of steps. All the equipment parts must be disinfected between sampling sites. After the disinfection, a plate from the chosen media needs to be placed in each of the six stages and the equipment must be properly closed to ensure a suitable sampling. The equipment has only a power switch, which means that the collection time must be controlled by the user depending on the number of litres needed, which is directly dependent on the expected contamination from the sampling site.	
Millipore air sampler	Millipore air sampler requires a small number of steps. The top part of the equipment (where the air passes) must be disinfected between sampling sites. After the disinfection, a plate from the chosen media needs to be placed into the equipment which must be properly closed to ensure the sampling. The equipment allows the choice of the number of liters needed which is dependent on the estimated contamination from the sampling location. The device automatically controls the time of the sampling collection.	
Surface swabs	Sampling with surface swabs requires the use of not only the swab, but also a square of known size, to limit a portion of the surface and make it possible to later calculate the densities per square meter. To facilitate the sampling collection, it is advisable to wet the swab in saline or distilled water.	
EDC	EDC have a very short sampling protocol since it is only necessary to place them in the sampling site. However, the cloth to be used has to be sterilized under UV light. It is recommended to attach the plate with tape to the collection site to minimize disturbance during the sampling period.	

Settled dust	This sampling method has an extraction protocol with a few steps, although it depends on the equipment used. The vacuuming filter needs to be properly attached to the equipment. The collection time must be controlled by the user, depending on the number of grams needed to perform the analyses. The filter and/or the dust (depending on the equipment used) must be stored in a sterile bag.	

The analyses performed followed the protocol already reported [24]. Surface samples were washed with 1 mL of 0.1% Tween 80 saline (0.9% NaCl) (orbital shaker: 250 rpm, 30 min). EDCs were previously weighed, and extracted using 20 mL of the same washing solution. As well as a piece (2 cm^2) of each settled dust filter, the sample is processed using 10 mL of the extraction solution. The dust collected by the vacuuming filters in each FFH was washed in a ratio of 1 g per 9.1 mL of 0.1% Tween 80 saline (0.9% NaCl) for up to 30 min at 250 rpm [4], and 150 µL of the washed suspensions from the passive methods samples were inoculated in two culture media (MEA; DG18). All samples were incubated during 5–7 days between 25 °C and 27 °C (MEA and DG18) [24].

Fungal densities were determined according to the sampling method used and applying the appropriate formulas, as performed by Viegas and team [4]. Morphological identification of fungal isolates was accomplished through macro- and microscopic features following [25] procedures and performed by the same mycologist.

2.2. Indexed Score Applied

A score was calculated from one (1) to three (3) for five (5) distinct sample parameters based on the characteristics stated above and satisfying the demands presented during the project: (a) accuracy—the number of different species found in each sampling method; (b) complexity field work—degree of complexity allocated to each type of collection equipment and materials needed, such as sample holders, swabs, specialized equipment, and personal protective equipment, as well as temperature for transportation and storage until further analyses); (c) cost—an estimated value per sample was calculated, based on current equipment and consumables; (d) complexity in laboratory work—the requirement for specialized or specially trained personnel for each analysis, with regard to the complexity for extraction and identification protocols and time consumption; (e) time taken since the fieldwork until obtaining the fungal contamination characterization—time required to obtain results from the field to the lab, considering the total process time for each sampling method until the final result (Table 2).

Table 2. Index of Scoring Criteria.

Sampling Methods	Criteria Application
Andersen six-stage	Considering the complexity of handling the equipment and the sampling, we determined that it requires training, and technical expertise, as it requires knowledge of the equipment's operation and purpose. In terms of the complexity of lab work and protocols, as well as the overall complexity of all work (from the field to the lab) and time consumption, it is a relatively easy sampling approach because all that is required after sampling is to incubate the plates before densities calculation and identification. In terms of cost, this sample method requires little processing material (plates and media), but it is dependent on the acquisition of equipment.
Millipore	As per the previous sampling method, the device employment will require training and technical skill because it demands an understanding of the equipment's operation and purpose. It is a remarkably straightforward sampling method in terms of lab work and protocols, as well as the overall complexity of all work (from the field to the lab) and time consumption, because all

	that is necessary after sampling is to incubate the plates before densities calculation and identification. Concerning costs, this sampling method is similar to the preceding method and only dependent on the equipment purchase, besides plates and media.
Surface swabs	The surface swabs technique is easy to perform. As in the other sampling methods, it demands knowledge of the sampling protocol. It is considered an accessible sampling approach since, while it does require an extraction protocol, it only has a few steps that take little time. In terms of cost, this sample approach involves the use of additional materials, but it is not dependent on equipment, and only needs some lab consumables and the extraction solution, besides plates and media.
EDC	This also needs comprehension of the sampling protocol and the specifics, and requires specific training. Since the EDC must stay in the sampling location for 15 to 30 days, the extraction protocol comprises several steps that take time and, considering the complexity of all the work (from the field to the lab), it was rated as a difficult sample method. This sampling method involves the use of additional lab materials (besides plates and media) and consumables (extraction solution), but it is not reliant on equipment.
Settled dust	This sampling method also requires training. Regarding the field and lab work after collection and the time required, it was considered more difficult than the other passive sampling methods employed. Concerning costs, this sampling method needs less specific equipment which is less expensive than the equipment used in active sampling, besides lab materials and consumables (extraction solution).

The analyses presented in this study took into consideration the index developed by the authors, based on personal and professional expertise as well as the experience and results obtained during the project. Table 3 represents the obtained score of each analyzed criterion previously described.

Table 3. Average scores of the five selected criteria for all the sampling methods employed.

Independent Variables	Andersen Six-Stage	Millipore Air Sampler	Surface Swabs	EDC	Settled Dust
Accuracy	3	3	2	3	2
Field Work Complexity	2	2	3	3	3
Economic Cost	1	1	3	3	3
Lab Work Complexity	3	3	3	1	1
Temporal Variation for Results	3	3	2	1	2
Total Score	2.4	2.4	2.6	2.2	2.2

2.3. Statistical Analyses Performed

Data were analyzed in IBM SPSS Statistics 28.0 software for Windows. The results were considered significant at a 5% significance level. For the characterization of the sample, frequency analysis (n, %) was used for qualitative data, and for quantitative data the minimum, maximum, mean, and standard deviation were calculated.

To identify the regressors of the "number of species", Poisson regression was used, with the "sampling site", "sampling method", and "culture media" being considered as independent variables. Since the variables are qualitative with more than two categories, dummy variables were created in order to allow for data analysis. To choose the best model, the Akaike Information Criterion (AIC) was used, and the best model is the one with the lowest value. To evaluate the quality of fit of the models, the quotient criterion Deviance/(n-k-1) was used, where "n" is the number of observations, and "k" is the number of groups. Regarding the residuals, for each model, the mean was calculated (which must be null) and it was evaluated if it presented constant variance.

3. Results

3.1. Score Criteria Results

To calculate the score, the following criteria were evaluated (accuracy, complexity in fieldwork, cost, complexity in laboratory work, and temporal variation for results), and values were assigned from 1 to 3, with 1 representing the worst performance and 3 representing the best performance in each of the categories. A total score value was generated for each sample by calculating the average of all the criteria. The accuracy for the highest fungal species diversity was obtained from Andersen six-stage, followed by Millipore air sampler, and EDC. The total score was obtained by mean of all the criteria (Tables 4 and 5). The higher final score was 2.6 for: surface swabs, and the lowest was 2.2 for settled dust and electrostatic dust cloths (EDC) (Table 5).

Table 4. Models Description: Identification of the Best Model.

Model	Independent Variables	|Devience/(n-k-1)|	AIC	Omnibus Test			Model Effect		
				X^2	Degrees of Freedom	Sig.	X^2 w	Degrees of Freedom	Sig.
Model 1	Sampling Site	1.102	473.654	7.053	8	0.531	7.153	8	0.52
Model 2	Culture Media	1.092	440.014	0.194	1	0.659	0.194	1	0.659
Model 3	Sampling Method	0.56	409.997	48.711	4	<0.001	46.717	4	<0.001
Model 4	Sampling Method + Sampling Site	0.527	446.942	55.764	12	<0.001	46.717 7.153	4 8	<0.001 0.52
Model 5	Sampling Method + Sampling Site + Culture Media	0.531	452.248	55.958	13	<0.001	46.717 7.153 0.194	4 8 1	<0.001 0.52 0.659

Table 5. Model 3: Identification of the Best Sampling Method.

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Testing			Exp(B)	95% Wald Confidence Interval for Exp(B)	
			Lower	Upper	Wald Chi-square	df	Sig.		Lower	Upper
(Intercept)	2.140	0.0808	1.982	2.299	700.722	1	0.000	8.500	7.254	9.959
Andersen six-stage	0.129	0.1108	−0.089	0.346	1.347	1	0.246	1.137	0.915	1.413
Millipore air sampler	−0.294	0.1237	−0.537	−0.052	5.656	1	0.017	0.745	0.585	0.950
EDC	−0.376	0.1267	−0.625	−0.128	8.825	1	0.003	0.686	0.535	0.880
Surface swabs	−0.687	0.1397	−0.960	−0.413	24.149	1	0.000	0.503	0.383	0.662
Settled dust	0 [a]							1		
(Scale)	1 [b]									

Dependent Variable: Number of Species. Model: (Intercept), Sampling Method. a. Set to zero because this parameter is redundant. b. Fixed at the displayed value.

3.1.1. Fungal Species Diversity

Regarding fungal diversity, the highest variety was obtained from Andersen six-stage samples where 27 and 22 species were identified on MEA and DG18, respectively. Through air sampled by Millipore 19 species were observed on both MEA and DG18. Both devices scored 3 in the accuracy criteria. Among the passive sampling methods, EDC had the highest number, having an accuracy score of 3, with 18 species being reported on MEA and 15 species on DG18. In samples from surface swabs, 10 species were detected on MEA

and seven species on DG18 (accuracy score: 2). In settled dust samples, seven species were identified on MEA and 12 species on DG18 (accuracy score: 2) (Figures 1–3).

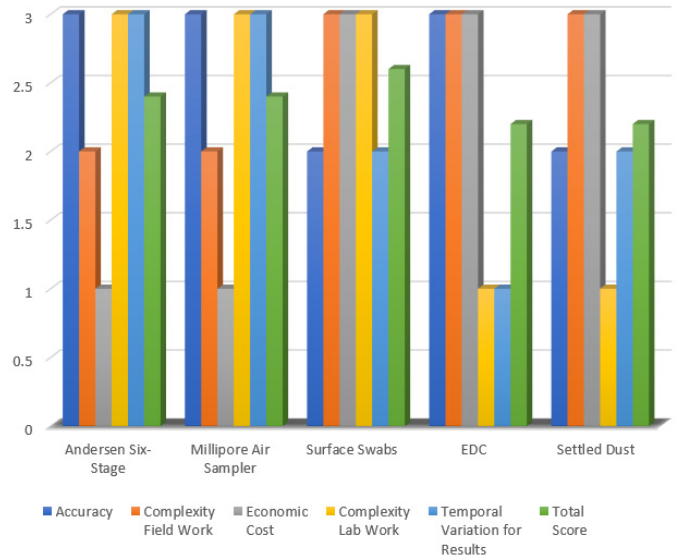

Figure 1. Average scores of the five selected criteria for all studied samples.

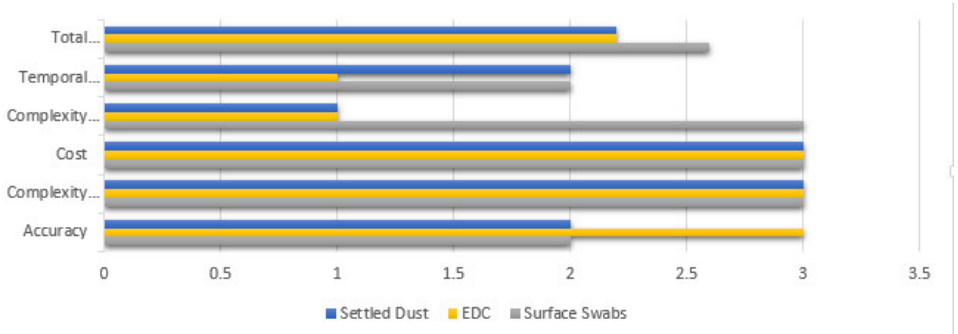

Figure 2. Average scores between passive sampling methods.

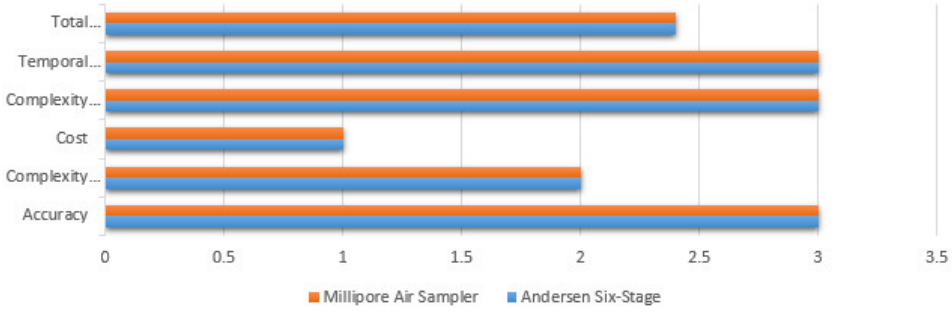

Figure 3. Average scores between active sampling methods.

3.1.2. Complexity in Field Work

Regarding the complexity of sampling, the use of impact samplers was considered to have the highest complexity, since they are dependent on the correct handling and operation of the equipment. Thus, the score value attributed was 2 regarding its performance, followed by settled dust collected from filters, surface swabs, and EDC which scored 3 points (Figures 1–3).

3.1.3. Cost

The materials, consumables, and equipment required for each sample were evaluated in order to estimate the cost associated with each sampling technique. The sampling methods that are dependent on equipment have a higher value which will negatively affect the cost performance, namely Andersen six-stage and Millipore air tester (score: 1 for economic cost criteria). Less expensive materials and equipment are necessary for passive methods, thus, higher score values for cost criteria were associated with settled dust, EDC, and surface swabs (score: 3) (Figures 1–3).

3.1.4. Complexity in Laboratory Work

The complexity in laboratory work differs regarding the sampling method performed. The protocols can vary, with samples requiring extraction and additional steps to get the results. Hence, we stipulated a score value of 1 to 3, 1 being the sampling method requiring a more complex protocol (with increased number of laboratory procedures). Considering all the steps, a score of 1 was attributed to the EDC and settled dust sampling methods, since the procedures for extraction increase the amount of work and, therefore, the complexity in the lab. As for surface swabs, it is considered an easy-to-extract sampling method, and for that reason a score of 3 was attributed to this sampling method. Active sampling methods, such as impactors, allow particle separation directly into a preselected medium, and only require incubation after sampling procedures. Therefore, a score of 3 was also attributed due to the simplicity of laboratory work (Figures 1–3).

3.1.5. Temporal Variation for Results

The temporal variation to access microbial results varies depending on the sampling method used and assay performed. Therefore, we considered a score value of 1 to 3, 1 being the sampling method where more time is required when considering all the procedures to obtain results. Since EDC stay in the field for over 30 days, a score of 1 was attributed for the time taken since the field work until obtaining the fungal contamination characterization, followed by settled dust and surface swabs (score:2). Active methods produce results faster when compared with the passive methods used, hence they scored highest (3 from 3) (Figures 1–3).

3.2. Statistical Analysis Results

Regarding the use of the independent variables as predictors for the number of species, it was possible to acknowledge that the "Sampling Site" (sig. = 0.52) and the "Culture Media" (sig. = 0.659) when applying MEA and DG18 are not statistically significant, which means that those variables do not influence the number of species. However, the variable "Sampling Method" (sig. < 0.001) is statistically significant, which means that the sampling method used can influence the number of species. Additionally, the model where the independent variable is the "Sampling Method" is the one with the lowest AIC (409.997) and, therefore, the chosen one to be applied (Table 4).

Once the model was chosen, the same was applied to the data and it was possible to conclude that settled dust, Millipore air sampler (B = −0.294), EDC (B = −0.376), and surface swabs (B = 0.687) would decrease the possibility of a high number of species, while Andersen six-stage increases the possibility of a high number of species. Additionally, this

trend is not statistically significant regarding settled dust, which means that both methods show a trend for a higher number of species (Table 5).

Additionally, in Model 5 (Table S1) that joins the three independent variables, "Sampling Site", "Culture Media", and "Sampling Method", it is possible to highlight that the results are identical to the models for each variable (Tables S2–S4—Supplementary material) (Table 5), respectively. Therefore, it can be guaranteed that, regardless of the sampling site or growing media used, the best sampling methods remain the settled dust and Andersen six-stage.

4. Discussion

To characterize occupational exposure to culturable fungi, active and passive sampling methods should be used [16,26]. Although personal or stationary sampling of airborne bioburden (comprising bacteria and fungi) can be applied to estimate exposure by inhalation, environmental variables, such as seasonal change and ventilation, can have a significant impact [16,27].

Settled dust and Andersen six-stage were the sampling methods that allowed us to identify a higher number of species and were also considered the best sampling methods to assess occupational exposure to fungi at FFH. In fact, both methods have been applied in different studies as stand-alone methods [16], or in parallel with other sampling methods [24,28].

Settled dust is considered a passive sampling method that has been described as being more repeatable than active sampling procedures in terms of anticipating microbial levels in indoor air [8,19]. It has also been documented as an environmental support for bacterial development, making it a bacterial contamination reservoir [8,29]. Depending on the location and surroundings of a workplace or facility, as well as the occupants and their activities, dust contains a wide range of components in various proportions [30]. However, in this case, the sampling site was not statistically significant as a predictor variable.

Active sampling methods for air collection, such as Andersen six-stage and Millipore, rely solely on culture-based methods, which can have advantages and disadvantages. The inflammatory and/or cytotoxic potential can affect the microorganisms' viability [6,12,31] which makes this method beneficial since it allows us to rely on the microbial composition to draw conclusions regarding the inflammatory potential variation [32,33]. In addition, it was already extensively reported that the health effects are mostly dependent on the fungal species found indoors [34–39].

In impaction sampling devices, a specific flow rate (depending on the type of environment) is defined to collect particles [8,40], by using its inertia to drive deposition on a collection media by promoting particle separation through an air stream [41,42]. However, since it only allows us to evaluate culturable microorganisms, the microbial load can be under-estimated due to the high velocity of the air flow that may result in microorganisms' cell damage and, consequently, the counts under-estimation [8,22,43].

Furthermore, as previously mentioned, air samples are only able to represent the load for a limited period of time [20] (usually minutes), whereas passive sampling methods allow us to collect the load from a longer period of time (days, weeks, or months) [16,21,44,45]. However, although active sampling methods (air samples) have a smaller collection time than passive sampling (EDC, surface swabs, and settled dust) they were able to present a more diversified fungal contamination [24]. Therefore, to ensure a more precise assessment of occupational exposure to microbial contamination, as suggested by the results, passive and active approaches should be employed together [16,26,46]. Increasing the number of alternative sampling methods will expand and enrich the data, allowing industrial hygienists to undertake a more accurate risk assessment [16,20,26]. As a result, sampling indoors should include more than one type of sample [15,16,47,48], and settled dust should be included in sampling methods to estimate fungal contamination exposure [16,49]. Regarding the complexity of both field work and laboratory work, as well as cost, surface swabs present the best option. Additionally, surface swabs have other

advantages such as the improvement of the surveillance sensitivity in areas where contamination is thought to be minimal when combined with air samples [49]. Also, the need of surface analysis to supplement mycological air characterization is supported by the fact that 64.2% of sampling sites had different species on the surfaces than those found in the air [49].

5. Conclusions

Overall, the statistical analysis allowed us to conclude that settled dust and Andersen six-stage were the best sampling methods to perform the assessment of occupational exposure to fungi at FFH, when considering the number of species. As for the final score, the results showed that surface swabs were, overall, the best sampling method considering all the criteria. The results obtained for surface swabs highlight the low complexity of their processing combined with the fact that it is a low-cost sampling method. This study reinforces the need to use a wide array of sampling methods when assessing occupational exposure to fungal contamination to ensure an accurate risk characterization.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/atmos13071123/s1. Table S1—Model 5: Interception of three models (Sampling Methods + Sampling Site + Culture Media); Table S2—Model 1: Identification of the Best Sampling Site; Table S3—Model 2: Identification of the Best Culture Media; Table S4—Model 3: Identification of the best sampling methods.

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Conflicts of Interest: The authors declare no conflict of interest. I have full control of all primary data and permission is given to the journal to review the data if requested.

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