

Common sampling techniques for the assessment of indoor fungal growth

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Abstract

The assessment of indoor fungi has recently gained increasing attention, both by the public and researchers, as the negative effects of the overgrowing moisture and mould-related issues have become more apparent. In order to detect the pathogenic and fabric-damage potential due to indoor fungal contamination, multiple air sampling methods were developed over the past decades. However, the selection of the most appropriate techniques for the quantification and/or the identification of the indoor fungal biota may still be challenging for inspectors. This paper aims to produce a critical summary of the most common air sampling methods available to date and underline the merits and risks of each method. The importance of the investigation's aim in the sampling technique selection process is highlighted.

Peer-review under the responsibility of the organizing committee of the ICMB23.

Keywords: fungi, mould, air sampling, investigation, assessment

1. Introduction/Background

Air sampling methods are widely used to identify and quantify airborne fungi contamination [1]. While surface sampling can be a powerful tool to assess localised fungal burden on surfaces, air sampling methods can provide helpful and holistic insight into both the diversity and the biomass of the fungal communities present in a room. However, though several air sampling techniques are available to date, the selection of the most appropriate method can be confounding due to the differences in terms of efficacy and cost, as well as technical expertise training they require. This paper sets out to produce an overview of the most common techniques and their merits and disadvantages, with the aim of providing practitioners and researchers with a rough guide for the selection of the most appropriate sampling technique according to the needs of their research.

2. Critical review of sampling techniques

Air sampling is considered to be the most robust way of assessing indoor fungal burden. Although a large number of sampling methods are available, only a few were widely used in the literature. In this study, a review of recent guidelines and research papers was carried out to identify the most common techniques and to determine their key characteristics (Table 1).

a. Impaction: Air sampling through impaction involves the recovery of particles onto a solid (e.g. adhesive-coated surface) or semi-solid (e.g. agar) collection medium [2,3]. Through an array of nozzles, a jet is created leading to the deposition of particles on a sampling medium that lies perpendicular underneath them. A significant pressure drop between the inlet and outlet of the nozzles leads to an increase in the particles' velocity. Due to the high velocity developed, particles with high inertia are being impinged on the sampling medium as they are unable to follow the airflow streamlines. On the other hand, low-inertia particles can escape capturing for being subjected to centrifugal forces that are not sufficient enough to force their deviation from the streamlines.

b. Liquid impingement: Particles enter the sampling device and are channeled through nozzles in a liquid medium that lies underneath them. Due to their inertia, large-size particles impinge in the medium. On the other hand, particles with low inertia can follow the airflow streamlines and escape capture.

c. Filtration: This technique relies on the recovery of particles onto a porous medium. Particles are forced to pass through the medium due to the airflow created. Due to their characteristics, such as size, shape etc., the size of the filter pores and the velocity of the air, large particles are unable to exit the porous medium while small-sized particles escape capture. Mechanisms such as inertial forces, diffusion and electrostatic attraction contribute to the collection of larger particles on the medium and the removal of smaller ones from the filter.

d. Electrostatic precipitation: Electrostatic precipitation relies on the collection of charged particles through the application of electric force. Bioaerosols are charged at the inlet of the precipitator. The particles are then exposed to an electromagnetic field that forces them to impact onto charged plates. The velocity of impact remains relatively low in comparison to impaction leading to less stress of microorganisms and thus losses of viability.

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e. Sedimentation / Gravitational settling: A sampling medium, usually agar, is placed within the space to be examined for a prolonged period of time. Due to indoor air currents particles collide and get impinged in the medium. Due to their inertia and gravitational forces, large particles may be collected easier than small-sized ones which may be carried by the indoor airflow and thus escape capture. The specific is able to provide only limited data related to fungal activity in the space under examination and may not be suitable for indoor fungal growth assessment.

Table 1. Common sampling techniques – merits and risks

Sampling method	Merits	Risks
Impaction	<ul style="list-style-type: none"> ++ Can be used to provide data regarding the viability of the detected fungi [4]. ++ Easy to use [5]. 	<ul style="list-style-type: none"> -- Prolonged sampling times can lead to desiccation, hence reduction of the collection efficiency of the sampler [3,6] -- Reduction of culturability can occur if high air flow rates are selected due to the subjection of particles to shear stress [4]. -- Bio-efficiency losses can occur due to desiccation. -- Particle bounce, and deposition build-up may reduce the collection efficiency of the sampling device [3].
Liquid impingement	<ul style="list-style-type: none"> ++ Can be used to avoid recovery losses due to desiccation [3]. ++ Higher number of microorganisms can be collected in the liquid medium and longer periods for sampling can be selected, in comparison to impaction methods [7]. ++ Both viable and non-viable microorganisms can be collected [7]. 	<ul style="list-style-type: none"> -- Losses in the collection efficiency may occur due to adhesion of particles on the inner walls of the liquid impinger [7]. -- Evaporation of the liquid and re-aerosolization of particles may lead to bioefficiency losses. -- Creation of violent bubbling due to the airflow created, can lead to re-aerosolization of particles, hence recovery losses [2].
Air filtration	<ul style="list-style-type: none"> ++ Easy to use [4,5]. ++ Samples can be used to detect both viable and non-viable fungi 	<ul style="list-style-type: none"> -- Recovery losses may occur due to stress from desiccation. -- May be difficult to analyse if the sampling period is short and the quantity of the particles collected is low
Electrostatic precipitation	<ul style="list-style-type: none"> ++ Particles impact the collection surface with low velocity [8] leading to reduced microorganism damage [9]. ++ Various collection media including agar, liquid or solid surfaces can be utilized [5] 	<ul style="list-style-type: none"> -- Desiccation in cases where long collection time is selected may affect viability of the microorganisms. -- Limited research available regarding the analysis of samples from electrostatic precipitation
Sedimentation (Gravitational settling)	<ul style="list-style-type: none"> ++ Easy to use. ++ No special equipment is needed 	<ul style="list-style-type: none"> -- Possible detection bias towards particles with higher inertia. -- The data collected rely highly on the velocity of the indoor air [10] -- Accurate quantitative results cannot be extracted. -- The procedure may only be able to provide data for a part of the total spectrum of the available airborne particles.

3. Discussion

Table 1 demonstrates that no method can be used as a panacea for all indoor fungal tests. While methods such as sedimentation and impaction might be easier to implement, the limited data they provide pertaining to non-viable fungi make them unsuitable for assessments aiming to capture the full spectrum of indoor fungal diversity. On the other hand, methods utilizing filters, liquid impingers, and electric precipitation might need more resources, and specialized training and analysis to produce the desirable results. The selection of the most appropriate sampling technique should (a) allow the collection of particles conforming with the aims of the investigation (quantitative results, species diversity, identification of culturable fungi, etc.) and (b) maximize the collection efficiency of the particles that are critical for the analysis technique selected. The sampling method must also be chosen, keeping in mind the effect of the pre-environmental settings on the particle recovery efficiency [10,11]. Activated protocols that involve mechanical resuspension of particles prior to sampling was shown to be beneficial for techniques such as filtration that require high sampling periods for the collection of an adequate number of particles but might lead to an overestimation of the fungal burden in cases impaction methods are used.

References

- [1] Méheust, D., Le Cann, P., Reboux, G., Millon, L., & Gangneux, J. P. (2014). Indoor fungal contamination: Health risks and measurement methods in hospitals, homes and workplaces. *Critical Reviews in Microbiology*, 40(3), 248–260. <https://doi.org/10.3109/1040841X.2013.777687>
- [2] Grinshpun, S. A., Willeke, K., Ulevicius, V., Juozaitis, A., Terzieva, S., Donnelly, J., Stelma, G. N., & Brenner, K. P. (1997). Effect of impaction, bounce and re-aerosolization on the collection efficiency of impingers. *Aerosol Science and Technology*, 26(4), 326–342.
- [3] Haig, C. W., Mackay, W. G., Walker, J. T., & Williams, C. (2016). Bioaerosol sampling: Sampling mechanisms, bioefficiency and field studies. *Journal of Hospital Infection*, 93(3), 242–255.
- [4] Grinshpun, S. A. (2010). *Biological Aerosols*. In *Aerosols - Science and Technology*. (I. Agranovski (ed.)). Wiley-VCH.
- [5] Mainelis, G. (2019). Bioaerosol sampling: Classical approaches, advances, and perspectives. *Aerosol Science and Technology*, 0(0), 1–24.
- [6] Mainelis, G., & Tabayoyong, M. (2010). The effect of sampling time on the overall performance of portable microbial impactors. *Aerosol Science and Technology*, 44(1), 75–82.
- [7] Han, T & Mainelis, G (2012). Investigation of inherent and latent internal losses in liquid-based bioaerosol samplers. *J. Aerosol Sci.*, 45, 58–68.
- [8] Han, T & Mainelis, G (2008). Design and Development of an Electrostatic Sampler for Bioaerosols with High Concentration Rate. *J. Aerosol Sci.*, 39:1066–1078.
- [9] Zhen, H., Han, T., Fennell, D. E., & Mainelis, G. (2013). Release of free DNA by membrane-impaired bacterial aerosols due to aerosolization and air sampling. *Applied and Environmental Microbiology*, 79(24), 7780–7789.
- [10] Efthymiopoulos, S, Aktas, Y,D, & Altamirano, H. (2022) Mind the gap between non-activated (non-aggressive) and activated (aggressive) indoor fungal testing: impact of pre-sampling environmental settings on indoor air readings. *UCL Open: Environment Preprint*. DOI: 10.14324/111.444/000141.v3
- [11] Efthymiopoulos, S, Aktas, Y,D, & Altamirano, H. (2021) Air sampling and analysis of indoor fungi: a critical review of passive (non- activated) and active (activated) sampling. DOI: 10.14293/ICMB210021