Developing a methodology to assess mould growth hidden behind internal wall insulation.

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Abstract

Internal wall insulation is one of the few and possibly, the only feasible solution to efficiently reduce heat losses through the external walls of buildings where the application of external insulation is not an option, for example in conservation areas. However, the application of this intervention entails risks and may lead to unintended consequences, such as moisture accumulation and mould growth (1,2). Currently, no international standards and regulations exist to evaluate these hazards via non-destructive inspections. The present study aimed to design and propose a non-disruptive methodology for the assessment of mould growth in confined spaces such as air gaps and cavities within exterior constructions. The proposed method involved air sampling through impaction for the collection of data and culture-based techniques for their analysis.

Keywords: Internal Wall Insulation, Air Sampling, Air gaps, Cavities, Culture-based method

1.0 Introduction

The built environment accounts for more than a third of the primary energy demand in the UK (3). Solutions involving the improvement of the thermal envelope, especially the installation of insulation on uninsulated walls as a retrofit strategy for existing buildings, may contribute to enhancing the building's energy performance and to the reduction of greenhouse gas emissions.

Reducing heat loss through existing uninsulated walls can be achieved through the installation of external, internal, or cavity wall insulation. However, some options are not always applicable. Planning regulations, legislation, and the building's characteristics may restrict the application of some solutions. In the case of historic buildings, where the installation of external wall insulation may not be permissible by legislation, internal wall insulation (IWI) may be the only feasible solution. This intervention, however, might be accompanied by a risk of interstitial moisture and condensation. As a result, moisture-related problems such as mould growth may arise, leading to health implications and structural damage.

The present paper aims to assess the feasibility and implementation of a novel air sampling and culture-based technique for the identification of mould growing within confined spaces (interface of external walls and IWI). Towards this end, a literature review was carried out to provide an overview of the unintended consequences related to moisture accumulation and the sampling and analysis techniques currently used for the assessment of mould growth in the built environment.

Air sampling via impaction was used as a method for the collection of data concerning mould contamination, while culture-based analysis was applied for the analysis of the sampling data. A computational fluid dynamics (CFD) analysis was performed to examine the airflow pattern created by sampling air from a confined space. This analysis was complemented by a series of small experiments to help determine potential correlations between the sampling time, airflow rate, and quantity of mould within spaces such as air gaps. The experimental results were analysed, and the limitations and suggestions for future research discussed.

2.0 Literature

The installation of IWI may be one of the feasible solutions to reduce heat loss through uninsulated walls efficiently. This intervention, however, may lead to unintended consequences (1,4) such as condensation, moisture accumulation and mould growth (4).

Reduced ventilation or installation of materials with a lower water vapour permeability may be responsible for moisture getting trapped inside internally insulated walls (5). Interstitial condensation may also be the result of poor installation of equipment during the implementation of the intervention or the use of materials that have been exposed to humid conditions (6-8). Moreover, the inability to properly seal around penetrations and services may also be the cause of moisture accumulation (7). Changeworks (8) reports that trapped moisture may be attributed to thermal bridges that are created when the insulation of joist ends is improperly installed. Leaks from plumbing failures may also contribute to the aggregation of moisture within exterior walls (6, 8).

Mould growth and dampness can degrade wood-based materials (9) leading to expensive remediations (10). Health implications for occupants due to the emission of bioreactive agents and the damage of indoor materials can also be related to the appearance of mould growth and dampness. As reported by the World Health Organization (WHO) (11) damp indoor environments and the growth of mould may jeopardise occupants' health and disturb their wellbeing. Fungal fragments, spores, volatile organic compounds, and cells emitted by mould can cause health problems if they enter the human body through respiration (12,13). Studies by Caillaud et al. (14) and Fisk et al. (10) have found a causal relationship between mould growth and asthma in children. Furthermore, WHO (11) has reported the association between mouldy and damp environments and occupational asthma and allergic rhinitis. Moreover, allergic bronchopulmonary aspergillosis (ABPA), allergic fungal rhinosinusitis (AFRS), and hypersensitivity pneumonitis (though uncommon) may be attributed to exposure to mould. Allergic reactions with respiratory infections have also been linked to exposure to mouldy environments (11).

2.1 Mould growth assessment

An air gap of at least 3 cm between IWI and external walls has been suggested as a solution to avoid moisture accumulation and mould growth issues (15). However, this type of interventions may not always guarantee the prevention of dampness and the risk of mould growth (16,17). Hence, the assessment of risks related to mould should be of concern when evaluating the installation of IWI.

Various sampling methods and analysis techniques are currently available for the assessment and collection of microorganisms, spores, or fragments to identify mould species and determine the level of background contamination. Similarly, various international guides and standards describe sampling procedures and analysis techniques for the assessment of mould growth in the indoor environment, including Parts 16–20 of the BS ISO 16000 standard (18-22), EMSL's Microbiology Sampling Guide (23), and the ASTM D7338-14 standard (24). However, no legislation defining the identification of mould hidden between elements of exterior walls exist.

2.1.1 Sampling methods

Sampling methods are mainly divided into two main categories: (a) air sampling and (b) surface sampling. Verdier et al. (25) imply that the decision-making process regarding the selection of the most appropriate combination of sampling techniques and analysis methods should respond to the aim/s of the specific investigation.

a. Air sampling

Air-sampling methods are considered to be robust strategies for the determination of the characteristics and composition of moulds (26). Air sampling is often divided into two main groups: passive and active air sampling. Passive sampling relies on the collection of airborne fragments, spores, and microorganisms that pre-exist in the still air (27). On the other hand, active sampling or aggressive air sampling, as it is often referred to, involves the disturbance of the indoor air steadiness to achieve a resuspension of fragments and spores and thus increasing the concentration of airborne particles (28).

Air-sampling methods that have been widely used involve the collection of samples through impaction, liquid impingement, spore trap sampling, cyclone sampling, and air filtration. While air filtration relies on the collection of particles on a porous medium (filter) due to their size, all of the other methods mentioned involve the collection of fragments, spores, or microorganisms due to inertial forces. The selection of the most appropriate method relies on the data a particular study is attempting to acquire; however, every method has its limitations. The key characteristics of the sampling methods mentioned above are summarised in Table 1.

b. Surface sampling

Surface-sampling methods are utilised for the identification of mould activity on surfaces. These methods can be useful for determining whether mould contamination levels in the indoor environment are significantly affected by the growth of fungus on surfaces (28). Some of the methods that have prevailed over the past few years are

swab, wipe, bulk, and tape lift sampling techniques. However, the performance of these methods is often accompanied by air sampling procedures, and they aim to support the assessment of background contamination levels.

Categories	Sampling Method	Characteristics of the methods
Air Sampling	Impaction	Particles are collected from the airstream on a sampling medium due to their inertia Only culturable microorganisms that have been collected during the sampling procedure, can be identified.
	Spore Trap (Air) Sampling	Spores and particles are collected on a spore trap slide. Enumeration of total number of viable and non-viable fungal structures (Spores/m^3) or colony forming units (CFU/m^3) per cubic meter of sampled air can be achieved Identification of some types of moulds indoors can be achieved but should be be compared to the types observed outdoors.
	Cyclone Sampling	Particles are collected from the airstream, in a liquid due to their inertia. Prevention of desiccation of particles can be achieved Identification of both viable and non-viable particles can be achieved The method can be used for classification of particles
	Air Filtration	Fungi are collected on a porous medium (e.g. filter). Spores or CFU per gram of the sampled air can be measured.
	Liquid impingement	Particles are collected from the airstream, in a liquid due to their inertia. Prevention of desiccation of particles can be achieved Identification of both viable and non-viable particles can be achieved
Surface Sampling	Surface Wipe Sampling	Particles are collected, usually on a gauge. The method can indicate contamination levels.
	Surface Swab Sampling	Samples are collected by rubbing a surface. Utilization of swab sampling is preferable when other surface-sampling methods. The method can be used to indicate background contamination levels.
	Tape Lift Sampling	Particles are collected on tape. Identification of mould concentration in dust as percentage of mould in total dust can be achieved. The distribution of mould in the sample as percentage of each identified mould vs total spore count can be examined. The method can indicate contamination levels.
	Bulk Sampling	The method is considered to be destructive due to scratching, scraping, or coring of a surface to collect samples. The method can be used to indicate background contamination levels.

Table 1 – Categories and characteristics of existing mould growth sampling.

2.2.2 Analysis methods

Analysis techniques play a key role in the determination of the characteristics of the sampled mould and the accuracy and robustness of the extracted results. Parts 16 to 20 of the BS ISO 16000 guide (18-22) the processing of data of various sampling methods. However, it is important to mention that every technique has its limitations, hence using more than one technique can improve and maximise the accuracy of the assessment procedure (28). The WHO (11) has divided the analysis techniques into two main categories:

・*Culture-based methods*

These techniques require the cultivation of the culturable particles that were collected during the sampling procedure.

・*Non-culture-based methods*:

Non-culture-based methods rely on the analysis of sampling data that originates from either viable or non-viable particles that have been collected during the sampling.

The main characteristics of commonly used analysis techniques are summarised in table 2.

Analysis technique Categories	Analysis technique for interpretation of results	Advantages	Limitations	Comments
Culture-based methods	Image analysis process or manually.	The results can be used for quantitave and qualitative research. Many species can be identified	Non-culturable species cannot be identified	The cultivation of the particles (usually for 3–7 days) which have been recovered during sampling, on culture media, is required.
Non-culture-based methods	Microorganisms can be counted and identified by using microscopy, flow cytometry.	Identification of culturable and non-culturable species can be achieved Limited time is required for the extraction of results (in contrast to culture-based methods) Many microorganisms can be identified at level of genera and species	Validation of the results may not always be possible Components that are toxic or allergenic cannot be identified Identification of cell debris is not achievable The data analysis requires lab work and complicated procedures Advanced sampling methods are expensive	Non-culture-based methods do not require the cultivation of micro-organisms for the analysis of the data collected during sampling.
	Polymerase Chain Reaction (PCR) technique	High accuracy of the results Many species can be identified High sensitivity	Currently, limited reference for fungal sequence exist	
	Chemical and immunoassay technique	Identification of important fungal components and MVOCs that are associated with potential health issues, can be achieved	Mould species cannot be identified. Certain methods are expensive Possible identification of fungal components that are not associated with mould may occur	

Table 2 – Categories and characteristics of analysis techniques available mould growth examination

3.0 Methodology

The present study aims to develop a non-disruptive methodology for the assessment of mould growth within elements of exterior walls through impaction and culture-based analysis. First, a CFD analysis was performed to examine the flow pattern created during potential air sampling procedures in full-scale walls. The CFD analysis was complemented by small-scale experiments to understand the effect of the sampling time and airflow rate on the results of the inspection procedure.

A schematic representation of the setup designed for the implementation of the suggested sampling procedure is shown in Figure 1. An airstream was created by the pump, leading to the deposition of particles on the sampling medium within the sampling device (impactor). The volumetric flow rate during sampling was controlled by the adjustment of the pumps' backpressure according to the rotameter. To avoid sampling the outdoor air that may penetrate the wall through cracks, two holes were created from the internal side of the wall, which were positioned as defined by the CFD analysis.

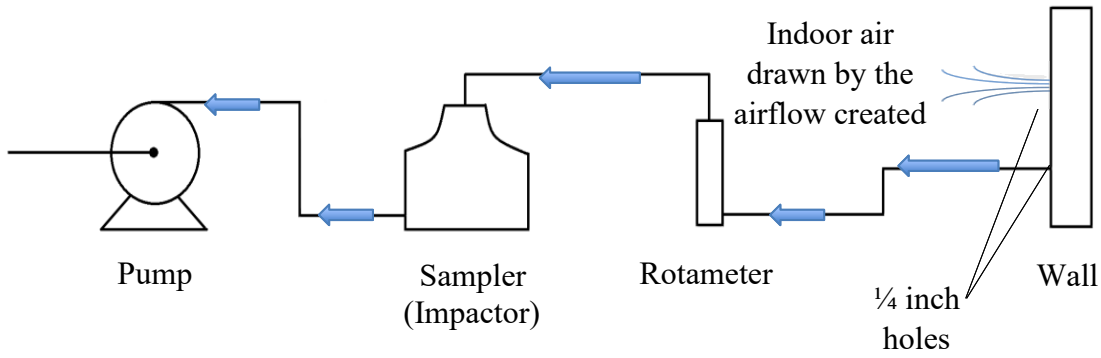


Figure 1 – Schematic representation of suggested experimental equipment for the assessment of mould hidden behind internal wall insulation

Impaction relies on the collection of particles on a sampling medium due to their inertia. Due to the airflow created by the operation of the pump microorganisms, spores and fragments enter the sampler and pass through the nozzles within it. An airflow jet is channelled through the nozzles and is deflected by the sampling medium that is placed horizontally underneath them (Figure 2). Mould particles with high inertia are unable to follow the airflow streamlines and attach to the sampling medium (agar). Particles with low inertia, on the other hand, follow the streamlines and do not get attached to the agar.

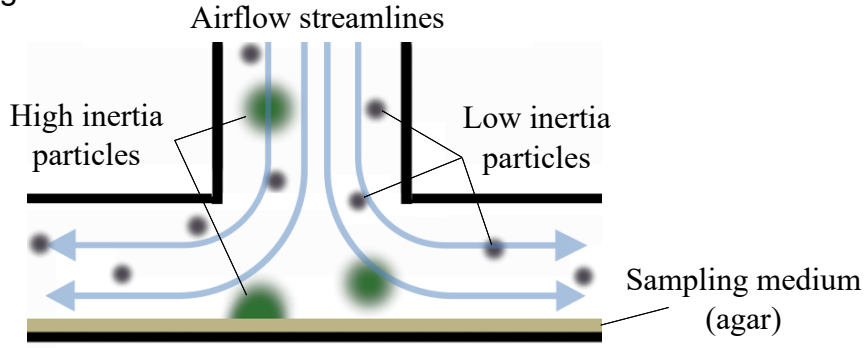


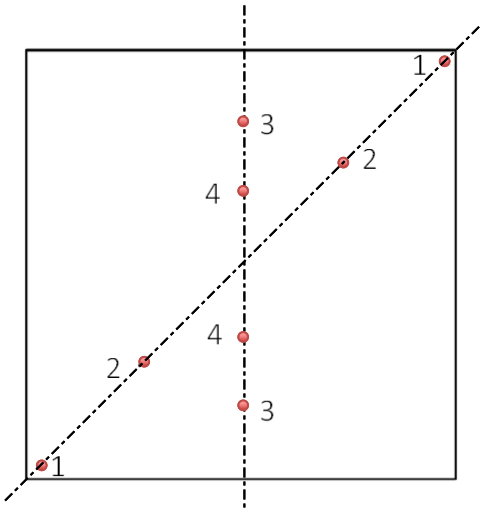
Figure 2 – Representation of the airflow created in an impactor

3.1 CFD Analysis

A short CFD analysis helped define the location of the holes. To create a geometry that best describes a full-scale wall, a 300 x 300 x 3 cm box was simulated in the CFD software. All surfaces of the simulated box were designed to be 2 mm thick. The internal space was considered to be still air at a pressure of 1 atm. Two ¼ inch holes were perforated in the surface of the box to represent the airflow inlet and outlet.

Four different locations for the holes were examined during this analysis, and these are represented in Figure 3. In cases one and two, the holes were located on a diagonal of the square surface, and the distance between them was set at 4 m and 2 m, respectively. The holes were placed on a bisector of the same surface in cases three and four and the distance between the two holes was 2 m and 1 m, respectively.

For the CFD analysis, assumptions and boundary conditions were determined. These are summarised in Table 3. Note that since the aim of the analysis was to develop a basic understanding of the airflow pattern, the velocity residuals levels were aimed to drop to a 1E-2 level. The velocity residuals calculate the imbalance of the velocity in all control volumes created through the modelling process and contain information about the convergence of the iterative solution.



Case 1: 4m distance between holes

Case 2: 2m distance between holes

Case 3: 2m distance between holes

Case 4: 1m distance between holes

Figure 3 – Representation of the location of the two holes in all four cases examined via a CFD analysis

It is important to mention that the temperature of the air through the CFD model was selected to be 15^{o}C. Taking into account that the simulations aimed to produce rough results regarding the airflow created during sampling, the effect of the temperature to the airflow pattern was not studied. Heat exchange phenomena were not taken into consideration for the purposes of this research.

Assumptions
The flow pattern was considered to not be affected by gravity. The flow was considered to be turbulent and as a result, the model that was considered to describe the flow phenomena best was the k-epsilon model. Transient flow phenomena and heat exchange were not taken into account. The mesh created through the simulation software consisted of triangles and was considered to be of good quality for the present analysis. For the convergence, a residual tolerance of 0.01 was considered for the velocity
Initial and boundary conditions
The fluid used for the simulations was air with a density of 1.225 kg/m^3 and a viscosity 1.7894·10-5 kg/m-s. No slip effect was considered to occur and the sides of the box were all considered to be stationary. The airflow rate at the outlet was considered to be 20 l/min. The pressure of the air at the inlet was considered to be 1 atm. The temperature of the air was selected to be 15^{o}C. Hybrid initialisation was utilised for the CFD analysis via ANSYS Fluent software.

Table 3 – Summary of all initial and boundary conditions along with the assumptions made for the CFD analysis

3.2 Experimental procedure

The *Aspergillus Versicolor* species, which has been reported to indicate an environment with excessive moisture BS ISO 16000-17:2008 (19), was cultivated for the experimental procedure on a total of 25 potato dextrose agar (PDA) plates. The mould was cultivated under optimum conditions (temperature of 27^o C and water activity of 0.98 (29)) for 14 days before conducting the experiments. Although the mould did not fully cover the plates, the growth rate of the *A. versicolor* was considered satisfactory for the experiments.

For the study, an apparatus was built consisting of a pump, a rotameter, an impactor, and a 45 x 45 x 5 cm box. The apparatus was utilised to conduct small scale experiments and to develop a basic understanding of the effect of the sampling time, volumetric flow rate, and size of mould coverage on the sampling results. The size of the box was specifically selected so that 25 Petri dishes (∅ 90) could fit inside it.

Two sets of experiments were conducted in an environmental chamber. Fans located on the ceiling of the environmental chamber operated during the first set of experiments; however, during the second set, they were turned off. The two series aimed to determine whether changes in the indoor air velocity influenced the sampling results. In each experiment, three different airflow rates (10 l/min, 20 l/min, and 28.3 l/min) and three different sampling times (1 min, 2 min, and 3 min) were tested.

4.0 Results and discussion

The airflow pattern for the four locations of the holes examined via the CFD analysis are depicted in Figure 4 below. From Figure 4 (a) it can be implied that the airflow created between the two holes may be strong enough to cause the resuspension of fungal particles and thus lead to a potential underestimation of the mould's

concentration in the sampled air. Furthermore, the results for cases two and three (Figure 4 (b), (c)) indicate that when the distance between the holes is 2 m, the airflow pattern, although altered, may not be appropriate for the assessment of mould growing between the holes. On the other hand, in case four (Figure 4 (d)), the airflow created seems to affect a large area near and between the holes. As a result, the orientation of the holes suggested in case four was considered the most appropriate for the assessment of mould growth in confined spaces such as air gaps. It is important to note that the significant differences in the flow patterns of Figure 4 could be attributed to the large changes in the distance between the holes.

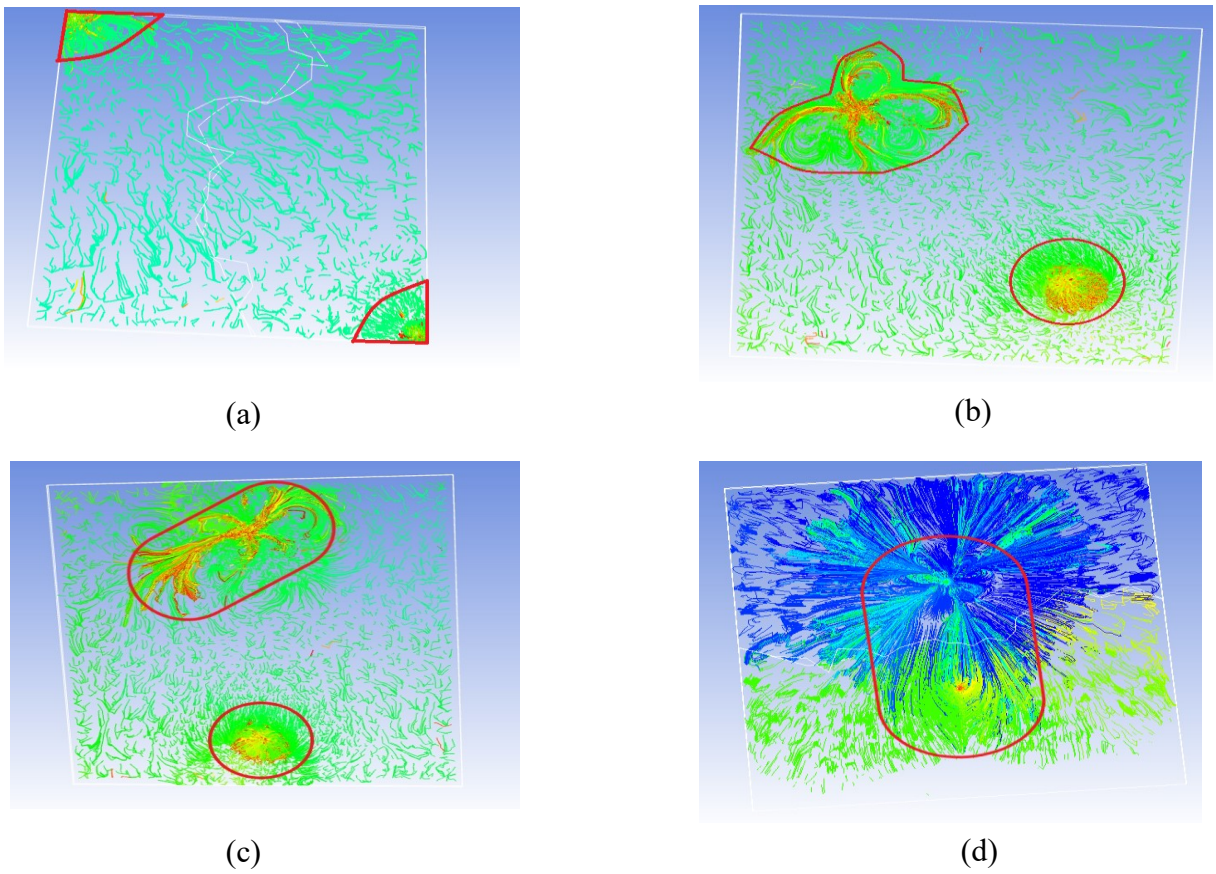


Figure 4 – **Representations of the airflow pattern for the four cases examined via the CFD analysis – (a) case one, (b) case two, (c) case three, and (d) case four**

According to the CFD analysis, placing the two holes on the bisector of a 3 x 3 m wall with a distance of 1 m between them seems to be the most suitable location for the assessment of mould growth within confined spaces. However, the assessment of mould growing relatively far away from the holes may prove to be difficult. Efthymiopoulos et.al. (30) implied that the location of mould within a confined space, such as an air gap may affect the results of air sampling via impaction. The performance of at least two air samplings using both holes as an inlet and outlet may help better understand the location of the mould and may prove to be a satisfactory solution to the issue.

Three sampling times (1 min, 2 mins, and 3 mins) and three sampling airflow rates (10 l/min, 20 l/min and 28.3 l/min) were tested for each set of experiments. The airflow rate remained constant (20 l/min) during the set of experiments where the effect of sampling time on the sampling result was examined. On the other hand, during the experiments for the determination of the effect of the flow rate on the sampling results, the sampling period remained 2 min. The results of the two sets of experiments are depicted in Figure 5.

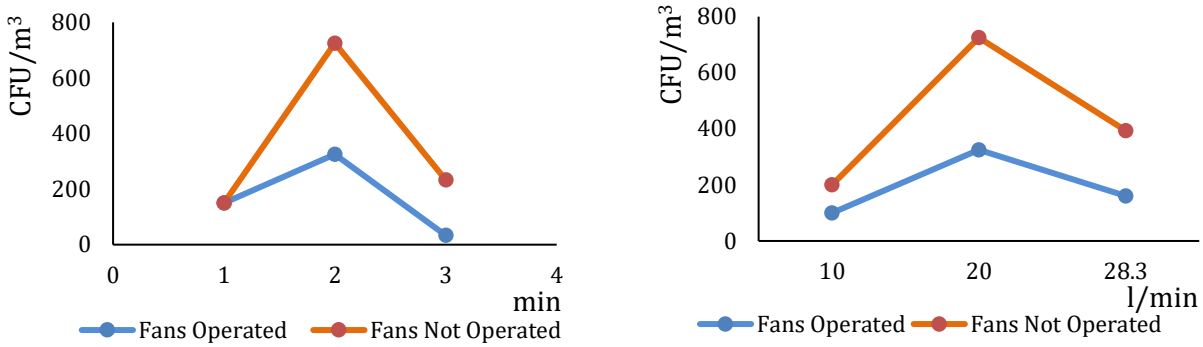


Figure 5 – (a) Comparison of CFU concentration (CFU/m3) from the experimental box for different sampling periods and (b) comparison of CFU concentration (CFU/m3) from the experimental box for different sampling airflow rates.

As shown in Figure 5 (a), among the three sampling times that were tested during the experiment, sampling for 2 min led to the highest number of colony-forming unit (CFU) per cubic metre of sampled air. A decrease in the CFU/m^3 was observed in the cases where the sampling time was 3 min. This reduction may be connected to a potential reduction in particle recovery due to the prolonged sampling time, as implied by Mainelis and Tabayoyong (31) in their study.

From the results presented in Figure 5 (b), it can be inferred that a higher CFU/ m^3 will be measured if an airflow rate of 20 l/min is selected. The CFU/ m^3 was found to be lower for the cases where the airflow rate was 28.3 l/min even though that is the specific airflow rate recommended by the manufacturer of the impactor. When using Versa traps by the same manufacturer, Adhikari et al. (32) found that a higher particle recovery was achieved when an airflow rate lower than the suggested rate was selected. The selection of high airflow rates may lead to the underestimation of the mould concentration in the sampled air due to the potential occurrence of particle bounce.

In both sets of experiments, it was observed that the operation of the fans in the environmental chamber led to lower values of CFU/m^3. The operation of the fans may have led to changes in the airflow pattern and consequently, may have increased the frequency at which the inertial impaction phenomenon occurred. Taking into account the fact that the space inside the experimental box is confined, an increase in the indoor air velocity may have led to the attachment of a higher amount of particles to the walls of the box or the tubing between the box and the impactor. Therefore, the sampling results of the cases where the fans were operated may have been underestimated. Similar results were extracted by Efthymiopoulos et

al. (30), who implied that the operation of fans and thus, the increased indoor air velocity might have contributed to the underestimation issue.

During the experimental procedure, higher values of CFU/ m^3 were measured when a flow rate of 20 l/min and a sampling period of 2 min were selected. The results of the present study may be related to the phenomena examined and described in studies by Adhikari et al., Grinshphun, Haig et al. and Thompson et al. (32-35). High air velocities may lead to a reduction of the culturability of sensitive microorganisms as they may be exposed to excessive shear stress (33). Prolonged sampling periods may lead to desiccation and thus, a reduction of the sampling device's bio-efficiency (32, 34,35).

In the experiments conducted the effect of relative humidity and temperature on the results of the sampling was not studied. Both variables remained constant during the experiment to avoid potential variations in the sampling results. It is important to mention that the examination of the effect of relative humidity to the results of the sampling may not always be clear. Thompson et al. (35) implied that during their experiments, a decrease in the recovery of a viable particle might have been attributed to the reduction of relative humidity from 90% to 30%. On the other hand, Ko et al. (36) considered that high relative humidity might be responsible for an increase of the aerosolized mould spore particles, which may have led to an increase of the inertial impaction frequency; therefore, the sampling results in their research may have been underestimated.

5.0 Conclusions

The present study was part of exploratory research aiming to examine whether air sampling through impaction and culture-based analysis could be used for the evaluation of mould growing in confined spaces such as air gaps between IWI and exterior walls. From the sampling procedure, other species apart from *A. versicolor* species were recovered. Species of the *Aspergillus* and *Penicillium* genus were also collected. Full identification, however, could not be achieved as microscopic analysis of the results was not performed.

The aim of the CFD simulations was the development of a basic understanding of the airflow pattern created in a real-scale, 300 x 300 x 3 cm air gap when a sampling flow rate of 20 l/min is selected. In the experiments the airflow created will not have the same pattern as the airflow created in real-life applications. As a consequence, some of the complexities of the phenomena taking place in real-scale scenarios may not have been incorporated.

As presented, the indoor air velocity, the sampling time and the flow rate may affect the sampling results. These effects may be connected to phenomena examined in earlier studies focusing on the bio-aerosol topic. Rapid changes of the airflow streamlines, due to increased velocity of the air inside the confined space, may have led to the increase of the inertial impaction frequency. Therefore, an increased number of particles may have been attached on the walls of the setup, resulting in the underestimation of the mould's concentration in the sampled air. Moreover, desiccation due to prolonged sampling times may have been responsible for the

reduction of the sampler's bio-efficiency and thus may have contributed to the underestimation issue. Reduced particle collection on the sampling medium may have also occurred, due to high air velocities. Grinshphun (33) implied that high air velocities might be responsible for the increase of particle bounce and thus may result in the reduction of particle collection. At the same time, the sampling results may have been underestimated due to exposure of particles to shear stress (34).

The results indicate that the assessment of mould growth through impaction and culture-based analysis may be feasible for the study. Creating two ¼ inch holes as designed, and selecting a sampling flow rate and period of 20 l/min and 2 min respectively, lead to a high recovery rate of mould particles. Repeating the sampling process more than one time using both holes as inlets, may also contribute towards the development of a better understanding of the mould's location. Moreover, measuring the indoor air velocity with an anemometer may provide useful information for the evaluation of the underestimation issue.

The present study was able to produce qualitative results regarding the effect of sampling time, flow rate and indoor air velocity to the sampling results. Conducting further research may lead to the development of a well-defined method to assess mould hidden behind internal wall insulation. Other aspects that may affect the sampling results (such as the size of the mould or the humidity and temperature of the environment) may be determined through the performance of additional small-scale experiments. The repetition of experiments may also lead to the extraction of robust results that, if synthesized with past and new findings could result in the standardization of an assessment method.

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