Airborne Fungal Particles From Growth In Buildings

The Influences of Dampness, Air Jets, and Air Transfer and Their Impact on The Inflammatory Potential

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BY
SOFIE MARIE KNUDSEN

DISSERTATION SUBMITTED 2017

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PREFACE

The studies that form the base of this PhD thesis were carried out during my employment at the Danish Building Research Institute (SBi) at Aalborg University in Copenhagen, as a part of the Skimmel.dk project. The work was conducted in the period from May 2013 to September 2017, including one year of absence for maternity leave. All laboratory studies were conducted during my external stay at the National Research Centre for Working Environment (NRCWE). Funding for this work was provided by The National Building Fund, The Danish Landowners Investment Fund, and Aalborg University.

First and foremost, I would like to thank my supervisor Professor Lars Gunnarsen for giving me this incredible opportunity and for your involvement in my project. Vast thanks to my co- supervisors, Senior Researcher Eva B Møller from SBi and Senior Researcher Anne Mette Madsen from NRCWE for your endless help and motivation. Thank you for believing in me - I am respectfully aware that I could not have completed this work alone.

Special thank goes to Margit W. Frederiksen for her great help and patience with me in the laboratory, and to Nadja Lyng for being a invaluable mentor from day one. I would furthermore like to thank my family, friends and wonderful collages at SBi and NRCWE, for their support and interest in my project. I would especially like to express my gratitude to Elvira Bräuner and Marie Frederiksen for their encouragement and support - and to Sirid Bonderup and Karen Bo Frydendall for always being ready to proof read my work.

Finally, my deepest gratitude goes to my wonderful husband Dennis, for your support and patience with me during this amazing journey - thank you for always looking out for me.

Copenhagen, September 2017

Sofie Marie Knudsen

ABSTRACT

Background: It has been acknowledged for centuries that fungal growth in buildings present a health issue, and over the years scientific evidence has established a link between staying in buildings with fungal growth or dampness and health related symptoms such as fatigue, headache, respiratory irritations, concentration difficulties, and even asthma and allergies. This makes it highly relevant to understand which mechanisms influence the concentration and species composition of airborne fungal particles in the indoor air. In environmental samples of airborne dust, the concentration of fungi is often more than 100 times lower compare to fungal aerosol samples generated in the laboratory. Thus, knowledge about the inflammatory potential of samples with low concentrations of fungi is limited. It is therefore important to obtain knowledge of the exposure risks from these more realistic exposure levels for the occupants.

Objective: The objective of this PhD thesis has been to obtain knowledge about how we are exposed to fungi in the indoor air as well as to investigate the inflammatory potential of samples containing multiple fungal species, released from gypsum boards. Thus, laboratory investigations were conducted of particles released from fungal infested gypsum boards. Furthermore, the inflammatory potential of the released fungal aerosols, were analysed in a bioassay with human granulocyte-like cells. In addition, simplified field measurements of the air transfer from attics and crawl spaces to their adjacent living areas were conducted, as well as measurements of the air change rate (ACR) in inaccessible construction cavities – e.g. wall and floor cavities.

Methods: The present PhD thesis is based on three published studies, in the following referred to as Paper I, II, and III.

In Paper I, the ACR in the construction cavities and adjacent living areas were measured using the tracer gas concentration decay method, and a simplified estimation of the air transfer from cavity to living area was made based on the constant concentration method. In Paper II, the amount of fungal particles released from fungal infested gypsum boards, and their aerodynamic diameter, were measured by an aerodynamic particle sizer connected to a particle field and laboratory emission cell. Low concentration fungal samples were generated from the infested gypsum boards and sampled on filter or in liquid by impingement. Both sampling time and air velocity were varied. Further, surface scraping and imprint plates were used for surface sampling of the fungal growth on the gypsum boards. All fungi were identified to species level by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. In Paper II, fungal species in samples of airborne dust from non-moisture damaged homes were divided into species associated with- and species not associated with mouldy buildings. These data were related to the ACR and relative humidity (RH) of the homes as well as the season during which the samples were

taken. In Paper III, the total inflammatory potential (TIP) of the fungal samples from the infested gypsum boards was analysed in a bioassay based on granulocyte like-cells.

Results: In Paper I, it was possible to measure an air transfer from crawl spaces to living areas. In one of the crawl space locations, it was possible to measure a build-up of tracer gas in the adjacent living area, which made it possible to estimate an air transfer by use of the simplified technique. For none of the investigated attic an air transfer to the adjacent living area was measured. In Paper II, the analysis of the species composition of the non-moisture damaged homes showed that season plays an important role, and that a higher ratio of fungal species associated with mouldy buildings was found in homes with higher RH and a lower ACR. Further, Paper II showed that the dried fungal infested gypsum boards released fewer fungal particles compared to the damp boards, which also had more substantial growth. In Paper III, the low concentration fungal samples from both the dried and damp infested gypsum boards induced an inflammatory potential in the bioassay. Further, a tendency to a J-shaped dose-response curve was found between the TIP and the fungal concentration of the samples.

Discussion and conclusion: For one of the crawl spaces, it was possible to estimate an air transfer, based on the simplified technique. Compared to attics, crawl spaces seemed to have a larger air transfer to the adjacent living areas.

The analysis of the species composition in the samples from the non-moisture damaged homes indicated that airing out is beneficial for the composition of fungal species in the indoor air. The analysis also showed that in cases where the species composition of the indoor air is used as a tool for determining the fungal burden of the building, the season during which the samples are taken should be taken into account. The dried fungal infested gypsum boards released fewer particles than the damp boards with more substantial growth, which indicates that immediate action in the remediation process has a beneficial effect on the occupants' risk of exposure to fungal particles in buildings.

The inflammatory potential of the low concentration fungal samples, from both dried and damp surfaces, indicate that drying a fungal infestation in a building is not sufficient to eliminate the fungal exposure risk for the occupants. The concentration used in the present PhD study was ranging in the curved part of the J-shaped dose-response curve, which weakens the conclusion on the influence of air velocity, dampness of the surface, sampling time, and sampling method on the TIP of the samples. However, it is an interesting finding in regard to interpretation of the results in future inflammation studies using environmental samples.

RESUMÉ (IN DANISH)

Baggrund: Vi har i århundreder vidst, at skimmelsvampevækst i bygninger er et problem, og gennem årene har videnskaben fundet en sammenhæng mellem det at opholde sig i fugtskadede- eller skimmelsvampeangrebne bygninger og helbredssymptomer som træthed, hovedpine, irritation af luftvejene, koncentrationsbesvær og endda astma og allergi. Det er derfor yderst relevant at forstå hvilke mekanismer, der har indflydelse på koncentrationen og artssammensætningen af skimmelsvampe i indeluften. I prøver af luftbårent støv fra indeklimaet er koncentrationen af skimmelsvampe ofte 100 gange lavere end i laboratoriegenerede prøver af skimmelsvampeaerosoler. Derfor er der begrænset viden om f.eks. det inflammatoriske potentiale af prøver med lave koncentrationer af skimmelsvampe. Det er derfor vigtigt, at opnå viden om eksponeringsrisici ved disse, for bygningsbrugerne, mere realistiske eksponeringsniveauer.

Formål: Denne Ph.d. afhandling har til formål at opnå viden om, hvordan vi bliver eksponeret for skimmelsvampe i indeluften, samt at undersøge det inflammatoriske potentiale af prøver med forskellige arter af skimmelsvampe frigivet fra gipsplader. Således er der udført laboratorieforsøg, hvor frigivelsen af skimmelsvampeaerosoler fra gipsplader med skimmelsvampevækst er undersøgt. Yderligere, er det inflammatoriske potentiale af de frigivne skimmelsvampeaerosoler studeret i et celleassay med humane granulocyt-lignende celler. Desuden er der udført simplificerede feltmålinger af luftoverførelsen fra krybekældre og loftsrum til tilstødende opholdsrum, samt luftskifte målinger i utilgængelige konstruktionshulrum, f.eks. hulrum i vægge- og gulvkonstruktioner.

Metode: Denne Ph.d. afhandling er baseret på 3 publiceret studier, der i det følgende kaldes for Paper I, II og III.

I Paper I blev luftskiftet i konstruktionshulrum og tilstødende opholdsrum målt og beregnet ved brug af sporegas og henfaldsmetoden, mens en simplificeret estimering af luftoverførelsen fra hulrum til opholdsrum blev lavet baseret på konstant koncentrationsmetoden. I Paper II blev antallet og den aerodynamiske diameter af partikler frigivet fra gipsplader koloniseret med skimmelsvamp, målt ved brug af en aerodynamisk partikelmåler forbundet til en *particle field and laboratory emission cell*. Prøver med lave koncentrationer af skimmelsvampe, blev genereret fra de angrebne gipsplader og opsamlet på filter eller i impinger væske. Prøvetagningstid og lufthastighed blev varieret. Derudover blev overfladeskrab og aftryksplader brugt til overfladeprøvetagning af skimmelsvampevæksten på gipspladerne. Skimmelsvampe blev identificeret til artsniveau ved brug af *matrix-assisted laser desorption/ionization time-of-flight mass spectrometry*. I Paper II blev skimmelsvampearter i prøver fra ikke fugtskadede hjem, opdelt i arter associeret med og arter ikke associeret med fugtige bygninger. Disse data blev sammenholdt med luftskiftet og den relative luftfugtighed

(RF) i hjemmene, samt den årstid prøverne var taget på. I Paper III, blev det totale inflammatoriske potential (TIP) af skimmelsvampeprøverene fra gipspladerne i Paper II, analyseret i et celleassay basseret på granolucyt-lignende celler.

Resultater: I Paper I blev der målt en overførelse af sporegas fra krybekældre til opholdsrum. Det var dog kun muligt for en af krybekældrene, at måle en opbygning af sporegas i opholdsrummet, der gjorde det muligt, at estimere en luftoverførelse ved brug af den simplificerede teknik. For ingen af de undersøgt loftsrum blev der mål forhøjede niveauer af spore gas i opholdsrummet. Paper II viste, at årstiderne spiller en vigtig rolle for artssammensætningen af skimmelsvampe i de ikke fugtskadede hjem samt, at der var en højere andel af skimmelsvampearter, der er associeret med fugtskadede bygninger ved højere RF og lavere luftskifte i hjemmene. Derudover viste Paper II, at udtørrede gipsplader, koloniseret med skimmelsvamp, frigav færre partikler i sammenligning med fugtige gipsplader, der var mere tilgroede. Paper III viste, at prøver med lave koncentrationer af skimmelsvampe fra både udtørrede og fugtige gipsplader, kunne inducerer inflammation i celleassayet. Derudover blev der fundet en tendens til en J-formet dosis-respons kurve mellem TIP og koncentrationen af skimmelsvampe i prøverne.

Diskussion og Konklusion: Det var muligt at estimere en luftoverførelse fra den ene krybekælder ved brug af den simplificerede teknik. Sammenholdt med loftsrummene havde krybekældrene tilsyneladende en større luftudveksling med de tilstødende opholdsrum.

Analyserne af artsammensætningen i luftprøver fra de ikke fugtskadede hjem tyder på, at det har en gavnlig effekt på artssammensætningen af indeluften at lufte ud. Yderligere viste analyserne, at i tilfælde hvor artsammensætningen af skimmelsvampe i støvprøver bruges til at vurdere belastningen fra skimmelsvampe i indeklimaet, så bør årstiderne for prøvetagning tages i betragtning. At de udtørrede gipsplader frigav færre partikler end de fugtige gipsplader, med mere skimmelvækst, indikerer at en hurtig indgriben i afhjælpningsprocessen vil have en gavnlig effekt på brugernes eksponeringsrisici, ved skimmelsvampepartikler i bygninger.

Det inflammatoriske potentiale af prøverne med lave koncentrationer af skimmelsvampe, fra såvel fugtige som udtørrede gipsplader indikerer, at udtørring af en skimmelsvampeskade i en bygning ikke er nok til, at eliminere bygningsbrugernes eksponeringsrisiko fra skimmelsvampeskaden. De koncentrationer af skimmelsvampe der blev benyttet i denne Ph.d. afhandling ligger i den kurvede del af den J-formede dosis responskurve, hvilket svækker konklusionen vedrørende indflydelse af lufthastighed, fugtighed af overfladen, prøvetagningstid og prøvetagningsmetode på TIP. Den J-formede dosis-respons kurve er dog en interessant opdagelse i forbindelse med tolkning af resultater i fremtidige studier af det inflammatoriske potentiale af prøver fra indeklimaet.

LIST OF INCLUDED PAPERS

This thesis is based on the following papers referred to in the text by the Roman numerals I-III.

I. **Sofie M Knudsen**, Eva B Møller and Lars Gunnarsen. *Assessment of exposure risk from hidden fungal growth by measurements of air change rates in construction cavities and living areas.* Journal of building physics, April 2017. DOI: https://doi.org/10.1177/1744259117704386

II. **Sofie M Knudsen**, Lars Gunnarsen and Anne Mette Madsen. *Airborne fungal species associated with mouldy and non-mouldy buildings – effects of air change rates, humidity and air velocity*. Building and Environment, Vol. 122, September 2017.

III. **Sofie M Knudsen**, Lars Gunnarsen and Anne Mette Madsen. *Inflammatory potential of low dose airborne fungi from fungal infested damp and dry gypsum boards.* Building and Environment, Volume 125, November 2017.

LIST OF OTHER SCIENTIFIC WORK

The following peer-reviewed publications have been conducted as part of the background research for the thesis. Their scopes are not aligned with the scope of the thesis and they are therefore not included.

- **Sofie M Knudsen**, Eva B Møller, and Elvira Bräuner. *Building characteristics that might determine moisture in 105 Danish homes*. Indoor Air 2014 conference proceedings. [1].
  Short summary:
  The aim of the study was to elucidate building characteristics and occupancy behaviour that increase risk of indoor dampness. Three methods of moisture assessment were conducted in 105 Danish homes, and building characteristics as well as occupancy behaviour were determined. Around 25% of the homes had moisture problems. Geographical location, drying clothe indoors, lack of mechanical ventilation, high social status, and spending more time indoors, significantly increased the risk of indoor dampness. Measurements of dampness in the indoor air reflected short-term variations in occupant behaviour, and measurement of dampness in materials reflected building characteristics.

- **Sofie M Knudsen**, Lars Gunnarsen and Anne Mette Madsen. *Fungal species present on and aerosolised from wet and dry gypsum boards.* The Danish Microbiological Society Annual Congress 2016, in proceedings. [2]
  Short summary:
  The aim of the study was to investigate what fungal species were found on the surfaces of fungal infested gypsum boards, versus what fungal species were recovered amongst the aerosols released from the infested gypsum boards. The gypsum boards were water damaged and inoculated with fungi sampled in a moisture damaged house. Fungal growth developed over a period of 6 weeks before half of the gypsum boards were dried out. The fungal aerosols were sampled on filer by GSP and by liquid impingement. Surface samples were obtained by gentle scraping of the infested surface with a sterilized scalpel. The results showed that there were a significant difference in fungal species found in the surfaces samples and the fungal species recovered in the aerosol samples from the gypsum boards. This indicates that a surface sample, from a fungal infested building, might not be a sufficient measure for what may be recovered in an air sample, and vice versa.

- Sirid Bonderup, **Sofie M Knudsen**, and Lars Gunnarsen. *Comparison of test methods for mould growth in buildings.* Climate 2016 conference Proceedings. [3].
  Short summary:
  The aim of the study was to compare test methods for assessment of fungal growth in buildings, and to evaluate if sampling of airborne fungi is an appropriate method for detecting hidden fungal growth and remediation needs. A case study was conducted to test the usefulness of different sampling methods available on the Danish market. In total four dwellings of different typology, with or without known fungal growth, was investigated. In each dwelling seven methods were used in parallel. The criteria for choosing the different methods were that they had to be non-destructive, frequently used by professionals, and relatively quick and easy. Two types of air samples indicated low levels of mould growth, even where the results of the other methods indicated high to moderate growth. In conclusion, we found visual inspection to be quite indicative of mould growth, while none of the surface tests gave the complete representation as stand-alone tests. The air sampling methods seemed only to react to very comprehensive infestation with fungi.

- Michal P Spilak, **Sofie M Knudsen**, Anne Mette Madsen, Barbara Kolarik, Marie Frederiksen and Lars Gunnarsen. *Association between dwelling characteristics and concentrations of bacteria, endotoxin and fungi in settling dust.* Indoor Air 2014 Conference proceedings. [4].
  Short summary:
  The objective of this study was to investigate possible associations between building characteristics and concentrations of airborne fungi, bacteria, and endotoxins. In total 27 dwellings were investigated, and characteristics such as; floor area, volume of the living room, flooring material, year of construction, floor level of the dwelling, and information about presence of pets were collected by researchers. The relative humidity, temperature, and concentrations of airborne fungi and bacteria were measured. The study showed a negative association between high floor area pr. person and bacteria and endotoxin concentrations in settled dust. Bacteria levels were also negatively associated with ventilation rate. Thus, increased ventilation rate might reduce the risk of exposure to high bacterial concentrations. Fungi were found in higher concentrations on higher floor levels, in rooms with larger volumes, and in homes with high relative humidity.

- Michal P Spilak, Anne Mette Madsen, **Sofie M Knudsen**, Barbara Kolarik, Erik Wind Hansen, Marie Frederiksen and Lars Gunnarsen. *Impact of dwelling characteristics on concentrations of bacteria, fungi, endotoxin and total inflammatory potential in settled dust.* Building and Environment 2015. [5].
  Short summary:
  Temperature, relative humidity, and ventilation rate weas measured in a total of 28 dwelling located in the Greater Copenhagen area. Dwelling characteristics including floor area, volume of the living room, floor material, year of construction, and floor level were gathered. The microbial exposure was measured by quantifying fungi, bacteria, and endotoxin concentrations in airborne dust. Further, the total inflammatory potential (TIP) of the dust was analysed. Higher concentrations of fungi were found in dwellings with high relative humidity, larger room volume, and in dwellings located on the second floor or higher. Higher concentration of bacteria was associated with small floor area per person and low ventilation rate. TIP was positively influenced by spring season, buildings constructed before the 20th century, and wooden floor. While TIP was affected by some dwelling characteristics, it was mainly influenced by season. In conclusion, people living in damp environments, with a small floor area pr. person are at higher risk of microbial exposure.

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CHAPTER 1. INTRODUCTION

A description of a remediation process for fungal growth in buildings can be found as early as the Old Testament in the Book of Levictus Chapter 14. This could possibly be the first mentioning, of how living in a building with fungal growth should be avoided. It should be noted that even back then, they did not advice people to just paint over the infested area, or in other ways cover it up. Since then, many studies have proven that these early observations are in fact true. Thus, today we likewise advise people to avoid fungal contaminated environments, and ensure careful remediation of buildings infested with fungal growth.

It is estimated that approximately 20% of buildings in Europe, Canada, and United States have signs of dampness [1, 6, 7]. Since we spend up to 60-90% of our time indoors [8, 9], and because remediation of fungal growth in buildings are rather resource-demanding, it is essential to develop tools to faster and better detect remediation needs of buildings with fungal growth. To do this we need a profound understanding of the mechanisms linking fungal growth in buildings, with the occupants' exposure to fungal particles in the indoor air.

1.1. OBJECTIVES

The aim of this PhD study has been to investigate the ways in which we are exposed to fungal growth in buildings, as well as to investigate the inflammatory potential of fungal samples containing multiple species. Thus, this PhD thesis covers laboratory studies of the release of fungal particles from infested gypsum boards, and studies on the inflammatory potential of the released fungal particles, measured in a bioassay using human granulocyte-like cells. In addition, simplified field investigations of ventilation rates in construction cavities and air transfer from the cavities to living spaces were conducted.

1.2. OUTLINE OF THE THESIS

The thesis consists of six chapters. Chapter 1 and 2 describes the motivation behind the thesis and covers explanatory background information. Chapter 3 describes the methodology used in both field and laboratory investigations. Chapter 4 describes and discusses the main findings of the three papers included in this thesis (Paper I-III). Chapter 5 provides conclusions based on the main findings of the thesis. Chapter 6 provides a perspective on the findings, and suggests the needs for further research. It should be noted that chapters 3-5 follow the chronological order of the papers.

1.3. HYPOTHESES

The hypotheses, which form the base of this thesis, are described in Figure 1-1. They are based on the main research question of the thesis, and divided into four overall hypotheses, named hypothesis I-IV, which are elaborated further in the lowest hierarchy of the figure. The dotted line boxes indicate in which paper the hypotheses are investigated.

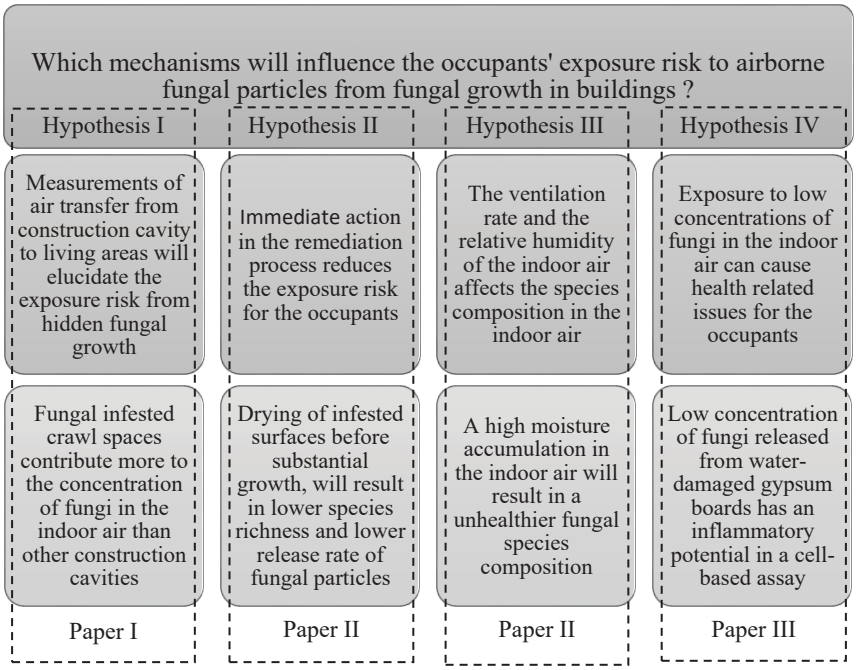

Figure 1-1. The highest hierarchy of the diagram is the main research question of the thesis, the middle hierarchy is the four main hypotheses of the thesis, and the lowest hierarchy is an elaboration of the four main hypotheses. The dotted line boxes indicate in which paper the hypotheses are investigated.

CHAPTER 2. BACKGROUND

Over the years, it has been widely established that moisture-damage and fungal infestation of buildings have significant health related consequences for the occupants [10-13]. Literature reviews, including studies from most parts of the world, concluded that there is an association between living in moisture- or fungal damaged buildings and symptoms such as, fatigue, headache, respiratory irritations, concentration difficulties and even asthma and allergies [7, 14-16]. Moreover, studies have found that many people spend the majority of their time indoors [8, 9], and that the frequency of buildings with moisture or fungal damage is rather high. E.g. a study found that 18% of the homes reported signs of dampness within the last 12 month [6]. Likewise, a review summarising ten publications concerning dampness in residential buildings, found that 12-24 % had reposted signs of dampness [7]. Thus, understanding the exposure risk from fungal growth in buildings is an important issue.

2.1. FUNGAL GROWTH IN BUILDINGS

If elevated moisture levels occur in building materials, fungal growth will likely be a consequence, since fungi can grow on most materials used in our buildings. However, four parameters are necessary to support fungal growth: damp conditions, nutrients, appropriate temperatures, and a latency period [17]. The optimal temperatures for growth of many fungi is between 25-30 °C, and in some cases the fungi can even grow at lower temperatures [18]. Thus a typical indoor environment will provide acceptable temperature conditions for fungal growth. Likewise nutrients will often be present in buildings, e.g. in terms of organic building materials or dust/dirt on the surfaces of inorganic materials [19]. Flannigan et. al. [18] describes how most fungi in the indoor environment are saprotrophs which obtain there nutrients from dead organic material and can live on even simple sugar or amino acid. In other words, the presences of nutrients in buildings and favourable temperature conditions are not easily avoided. Therefore, focus will often be on controlling the moisture levels in buildings [14]. Thus, understanding the mechanisms for elevated moisture in building constructions is strongly linked to prevention of fungal growth in buildings.

2.1.1. ELEVATED MOISTURE IN BUILDINGS

As stated in the PhD thesis by Hägerhed Engman [20] and in a book by Flannigan et. al [18], there are four main principals for elevated moisture levels to occur in building constructions. (1) water damage e.g. from a leaking installation part or a flooding, (2) penetrating moisture from the ground, (3) build-in moisture from the construction process, and (4) absorption of condensate moisture on the surfaces caused by a high water content of the indoor air – e.g. due to poor ventilation. In relation to moisture in buildings it is important to distinguish between air dampness, typically measured as

the relative humidity (RH) of the indoor air (% RH), and the moisture content of a material (weight-%), which is a measure for the moisture content relative to the weight of the dry material. A study including 105 Danish homes found that different factors influence these two types of dampness. E.g., occupancy behaviour related dampness was the associated with elevated moisture levels in the air, whereas building characteristics was associated with elevated moisture in the building structure [1].

2.1.2. FUNGAL GROWTH ON BUILDING MATERIALS

Moisture requirements for fungal growth on building materials are often expressed as water activity on the surface, or in % RH on the surface of a material in equilibrium with the surrounding air. The relationship between the two is expressed in the following equation:

$$\varphi = a_W \cdot 100 \qquad (1)$$

Where φ is the RH on the surface and a_W is the water activity on the surface [17].

Johansson et al. [21] describes how the moisture requirement for fungal growth depend on the building material (Table 2-1). It is suggested that a RH below ≈75 % RH on the surfaces will limit the risk of fungal growth [7, 21, 22].

Table 2-1. Moisture requirements for fungal growth on different building materials, based on literature review [21]. The values are based on long-term exposure at 20 °C.

Building materials	a_w requirements for fungal growth
Wood and wood based materials	0.75-0.80
Gypsum boards (sandwiching cardboard)	0.80-0.85
Mineral wool	0.90-0.95
Expanded Polyester (EPS)	0.90-0.95
Concrete	0.90-0.95
Materials with dust or dirt on the surface	0.75

Many studies of fungal growth on building materials have used gypsum boards as representatives [19, 23-28]. Gypsum boards are a typical used building material in both wall and ceiling constructions. Simplified, they consist of a gypsum core with a layer of cardboard sandwiching the gypsum core. As cardboard contains cellulose gypsum boards are sensitive to damp conditions, and fungal growth is therefore likely to happen on the cardboard surface [19, 29].

2.1.3. HIDDEN FUNGAL GROWTH

Fungi can grow visible on free and open surfaces in the building, or hidden inside the structure. Fungi that grow hidden inside a wall construction, underneath a floor construction, in crawl spaces or in attics, are often referred to as hidden fungal growth.

Over the years, composite constructions have become more widely used in buildings, and due to the complexity of these multilayer constructions, an increased risk of hidden fungal growth follow. A study of 219 Finish homes, found that 1/3 of the buildings had hidden fungal growth that required destructive investigations of the building structure to identify [30]. In the case of hidden fungal growth, the building structure behind which the fungi is growing will work as a barrier [31] and possibly limit the concentration of fungi in the indoor air. However, in many cases occupants still report symptoms [3, 14, 32]. In comparison to visible growth, it is necessary to conduct destructive investigations to determine the extent of the hidden fungal growth, and the remediation often requires restoration of larger parts of the building structure, thus leading to a more resource demanding remediation. Therefore gaining knowledge on the transport routes from hidden fungal growth in buildings, is necessary to better understand the exposure risk from the hidden fungal growth, and thereby optimize the remediation process.

2.1.4. DETECTION AND REMEDIATION PROCESS

The remediation process of fungal damages in building varies with the situations, but some overall steps can be defined. Figure 2-1 illustrates a typical remediation process divided into six overall steps. As the illustration shows, it is important in any remediation process first to identify and repair the source of the damage. Depending on the location and extent of the fungal growth, different cleaning methods are available, e.g. mechanical cleaning, cleaning by steam or cleaning by chemicals. In cases where cleaning is not possible the infested material should be replaced. When repairing a damage or replacing infested material, it is important to think the design of the new construction through, to avoid creating a new potential risk construction. Finally, a control of the cleaning and repairs should be conducted to ensure that the occupants are in fact no longer exposed. Many guidelines on the remediation process for fungal infestations in buildings likewise acknowledge these overall steps and stress the point of protection of the workers during the remediation work, and carefully sealing of the contaminated area to avoid contamination of other areas [18].

Figure 2-1. Illustration of a remediation process divided into six overall steps and with listed highlights for each step.

Detecting fungal growth in buildings can be more or less complicated depending on the location and cause - e.g. whether the fungal growth is visible or hidden. In the cases of hidden fungal growth, the detection process might be more comprehensive compared to visible fungal growth, and will often require destructive investigations into the building structure. To try to avoid destructive investigations, screening for hidden fungal growth is often done by sampling of fungal aerosols in the indoor air. E.g. a study found that 90% of the buildings with fungal growth in the wall cavities was detected by air sampling of fungal aerosols [33]. However, the study also conclude that air sampling, as a method for detecting fungal infestations in a building, often results in false negative observations - as other studies have likewise found [3, 34-37]. In addition, a study found that a visual inspection by professionals, was the most effective tool for detection of hidden fungal growth [3]. Further did another study find that the use of multiple assessment methods, would be necessary to best evaluate the microbial exposure risk of the indoor air [38].

2.1.4.1 Species composition as a tool for detection of fungal growth

A study has found that the fungal genera composition differs between reference buildings and buildings with frequent signs of moisture damage [39], indicating that knowledge on the composition of fungi in the indoor air might aid in the process of detecting hidden fungal growth in buildings. To facilitate the detection process of fungal infested buildings, an Environmental Relative Mouldiness Index (ERMI) has been developed in the US. ERMI is a tool for determining the fungal load of a building, and it consists of 36 fungal species, which are divided into two groups. Group 1 includes 26 species that are associated with fungal infested or damp buildings and group 2 includes 10 species that are often found in the indoor air, but not associated with fungal infested buildings [40]. The ERMI value is a measure for the fungal load of a building and based on the concentration of each fungal species in an aerosol sample [40]. A French study has found association between higher ERMI values and measurable fungal aerosols or visual indications of fungal growth in buildings [41], and studies across the US have found associations between higher ERMI values and asthma symptoms amongst both children and adults living in the buildings [42-45]. This indicates that the ERMI value might be useful to determine if the indoor air has a problematic composition of fungal species both in regard to water damage and health issues for the occupants. However, a Finish study suggests that the ERMI index should be modified according to geographical and climatic differences [46], and thus found that the ERMI index in its present state is not useful for identification of problematic buildings in the sub-arctic climate of Finland. With the development of methods that quickly can identify fungal species correctly [47], more knowledge on species level is to be expected.

2.1.5. SAMPLING METHODS FOR FUNGI IN BUILDINGS

Dust samples from either surfaces or indoor air, are often used to identify the extent of fungal growth in buildings, especially in the cases of hidden fungal growth. However, it should be borne in mind that there is not always a consensus between fungal growth on the surface of building materials and what is recovered in the indoor air [2]. Recently a study has identified seven of the most used sampling methods amongst practitioners in Denmark, two of which were air sampling methods [3]. Both air sampling methods showed a poor correlation with the other sampling methods in regards to determining fungal infestation of the buildings. The other methods were either active air sampling onto a filter or onto a growth medium, sampling by imprint plates or swipe samples of dust from surfaces. However, other methods for sampling of airborne fungi are available and commonly used in research. E.g. sampling of airborne microbes by use of Electrostatic Dust Collector (EDC), the GesamtStaubProbename (GSP) or the BioSampler SKC (impinger). All three methods have proven representative for sampling of airborne fungi, even though differences was discovered between them [48]. E.g., results from the impinger sampling in general show lower concentrations of microbes in the indoor air than the GSP sampling. These methods, especially the EDC, offers the opportunity to collect dust samples over a longer period than the commercially available methods used in [3], and might therefore create a more realistic measure of the concentration and composition of airborne microbes in the indoor air.

In the indoor air of buildings with no moisture or fungal problems, the concentration of fungi is typically $<10^3$ CFU/m^3 and seems only rarely to exceed 10^4 CFU/m^3 in buildings with visual fungal damage [49, 50]. However, understanding the exposure pathways for fungal growth in buildings is a complex matter, since many factors will influence the level of fungal aerosols in the indoor air. E.g. studies have found that the release of fungal particles from infested surfaces depend on parameters such as air velocity [23, 51, 52], dampness [53], and the fungal species growing on the surface [23]. Outdoor fungi also influences the indoor fungal concentration and species composition [54-57]. Several studies have found that the fungal concentration of the outdoor air is typically higher during summer than winter [58-62], and in non-mouldy buildings, the indoor concentration of fungal aerosols is likewise higher in summer than winter [56, 58, 60-62]. Thus, the indoor fungal concentrations seem to be affected by season [56]. Studies have also found that the fungal species composition of the indoor air is affected by season [58, 60]. This is important knowledge to keep in mind when evaluating if there is a risk of fungal infestation in buildings using measured airborne fungi or dust samples as a tool.

2.2. AIR CHANGE RATES IN BUILDINGS

Several studies have proven that the air change rate (ACR) influences the indoor air quality in different ways. E.g. is low ACR associated with increased levels of microbes [7, 56, 63] and infectious agents [64], with reduced productivity amongst the occupants [65, 66], with elevated moisture levels [7, 14], with sick building syndrome [67, 68], and with health issues such as respiratory diseases and allergies [63, 68]. Further, studies have found that mechanical ventilation (e.g. exhaust or inlet and exhaust in combination) will lower the concentration of moisture and fungi in the indoor environment [1, 69]. Thus, when evaluating the indoor air quality or risk to microbial exposure, knowledge on ACR is extremely beneficial [20, 32, 68]. In most European countries, national building codes include requirements for a minimum ACR of the indoor air. In Denmark the current building regulation requires a minimum ventilation rate of 0.3 l/s pr. m^2 [70], which is equivalent to 0.5 h^{-1} for normal sized living spaces. This requirement is in accordance with two review studies, which conclude that ventilation rates below 0.5 h^{-1} will increase the risk of allergies and asthma for the occupants [68, 71].

Tracer gas, both active and passive techniques, is commonly used for measurement of the ACR in buildings. Depending on the objective of a study, different tracer gas techniques are available. Table 2-2 describes the advantages and limitations of the most frequently used tracer gas techniques, concentration decay, the constant injection, the constant concentration, and the perfluorocarbon tracer (PFT, based on the constant injection technique). Conducting ACR and air transfer measurements in construction cavities that fit the requirements for hidden fugal growth, might be an efficient way to assess the contribution to the indoor air pollution from such cavities.

Table 2-2. An overview of the advantages and limitations of each of the four tracer gas methods [72-75].

Tracer Gas Methods	Advantages	Limitations
Concentration Decay	Often requires a short timespan for measuring even small ACR and uses less tracer gas pr. measurement than other methods.	Assumes a constant ACR during the measurement and is therefore sensitive to changes in the ACR during the measuring period.
Constant Injection	Useful in leaky environments, where the concentration decay method would result in a very short sampling time.	Before the measuring can start, the concentration must be at least 95% of the equilibrium concentration, thus making the measuring period rather long.
Constant Concentration	Useful to detect even small changes in the ACR and is therefore often used to conduct measurements in occupied rooms.	Requires rather expensive technical equipment compared to the other methods, and a significant longer measuring period than the concentration decay method.
PFT (based on the constant injection method)	Useful for measuring ACR over large timespans in occupied rooms.	Based on vapour droplets evaporating from small tubes, which possibly makes it less accurate when measuring in small cavities were the surface to volume ratio is large.

2.3. FUNGAL AEROSOLS AND INFLAMMATORY POTENTIAL

Aerosolisation of fungal particles from infested surfaces in buildings present an exposure risk for building occupants, in terms of inhalable fungal aerosols that might create inflammatory symptoms in the airways. Thus *in vivo* and *in vitro* studies have found that exposure to fungal samples from damp buildings causes inflammation [76-80]. Likewise, has an *in vitro* study found that fungal aerosols sampled in biofuels contributed to the inflammatory potential of the samples [81], and an another *in vitro* study using samples of microbe aerosolised from gypsum boards also found an inflammatory potential of the samples [53]. Further, *in vitro* studies found that the inflammatory potential of environmental samples from non-moisture damaged homes was mainly affected by seasonal variations of microbial levels in the indoor air [5, 56]. Environmental samples used in *in vitro* studies of inflammatory potential of airborne fungi, seem to contain lower concentrations of fungi ($< 10^2$ CFU (-or spores)/ml) [56, 82] than fungal samples generated in the laboratory (10^5-10^7 CFU/ml) [53, 81, 83]. This indicates that low dose exposure studies are relevant to extent current knowledge on the exposure risk to fugal aerosols in the indoor air.

CHAPTER 3. METHODOLOGY

This chapter provides information on the methods used in the present PhD study. The investigations of this study were conducted as a mix of field- and laboratory studies. In the field, the possibility of conducting simplified investigations of the air transfer from attics and crawl spaces to their adjacent living areas were made, as well as measurements of the ACR in construction cavities such as crawl spaces, attics, wall- and floor cavities, since such cavities often are subjected to hidden fungal growth [84]. In laboratory settings, investigations were made on the influence of dampness, air velocity, and sampling time on the release of fungal aerosols from water-damaged and fungal infested gypsum boards. Further, analyses were conducted investigating the total inflammatory potential (TIP) of the samples, which contained low concentrations of fungi (10^2-10^5 CFU/ml). In addition, analyses of the influence of ACR and RH on the species composition in non-moisture damaged homes were made, based on the ERMI Index.

3.1. ACR MEASUREMENTS (FIELD STUDY)

In the field study described in Paper I, ACR measurements were conducted in a total of 12 construction cavities and their adjacent living areas (Table 3-1). The concentration decay method was used for the ACR measurements. A detailed description of the locations can be found in Paper I.

Table 3-1. Locations used for measurements of air change rate and air transfer in Paper I.

Cavity type	No of cavities	Inaccessible	Accessible	Building type
Crawl space	7		+	Homes
Attic	2		+	Homes
Floor cavity	1	+		Home
Wall cavity	2	+		Home and office

Two types of tracer gases were used for the measurements, SF_6 and N_2O. The two chosen tracer gases both had rather low threshold limits (SF6=1000 PPM, N2O=50 PPM) [73, 85], and were both naturally present in extremely low doses in the indoor air (≈1 PPT for SF_6 [86], and ≈0.3 PPM for N_2O [87]). This meant that only small doses of the tracer gas was necessary during the measurements. The two types of tracer gasses were not used in parallel, meaning that the first set of measurements were carried out with the use of SF_6 and later measurements were carried out with the use of N_2O. This was due to available resources during the field investigations and because the use of N_2O tracer gas allowed for a much simpler design for dosing of the tracer gas. A small handhold unit loaded with a gas cartridge of 8 g N_2O made it considerably easier to control the dosing compared to the rather larger SF_6 gas bottle (>100 l).

In order to avoid layering of tracer gas due to differences in density, thorough mixing is necessary. A way to ensure a thorough mixing of the tracer gas and room air, is by mixing with running fans throughout the measuring period. However, this method present issues in terms of increased pressure differences, which might affect the ACR. Thus, in the present PhD study ACR measurements were carried out with and without constant mixing, depending on the cavity type. Generally, all ACR measurements of the living spaces and accessible cavities were carried out by use of constant mixing, whereas the measurements in the construction cavities were without constant mixing. For two of the locations (C1 and C2), the effect of the constant mixing was studied by repeating the ACR measurements without constant mixing in both living areas and crawl spaces (Paper I).

3.1.1. TRACER GAS DECAY

When conducting ACR measurements, it is important to pay attention to what would be the most beneficial technique for the individual measurements [74], and depending on the chosen method, conducting the ACR measurements can be rather comprehensive. The tracer gas decay method is amongst the most frequently used methods in practice, and require considerably shorter sampling time compared to other tracer gas methods (e.g. constant injection) [73]. In the present PhD study, all locations were unoccupied during the measuring period, and the ACR was therefore assumed steady throughout the period. Thus, the concentration decay method was suitable (Table 2-2).

The decay of tracer gas in the room or cavity will happen over time, following an exponential curve. Accordingly, the ACR was calculated by use of the decay regression [73, 88]:

$$C(t) = C \cdot e^{-\lambda \cdot t} \qquad (2)$$

C(t) is the tracer gas concentration over time [PPM], C is the initial tracer gas concentration [PPM], λ is the air change rate [h^{-1}], and t is time [h].

The INNOVA 1302 Photoacoustic Tracer Gas Monitor (Brüel & Kjær, Denmark) was used for measurement of the tracer gas decay. The gas monitor analyses the air samples in a small sampling chamber by use of optical filters [89]. Previous studies of ACR in residential buildings [90-92] and office buildings [91], have likewise used the INNOVA for measuring the decay of tracer gas. The gas monitor compensated for temperature fluctuations and water vapour interference during the measurements.

3.1.2. AIR TRANSFER

In addition to the ACR, the air transfer between cavity and living space was measured in a total of four location, two crawl spaces and two attics. Estimations of the air transfer from cavity to living area were made on the basis of the equation for build-up of tracer gas in a room using constant concentration [73]:

$$C_{eq} = \frac{E}{\lambda \cdot V_r}\left(1 - e^{-\lambda \cdot t}\right) \qquad (3)$$

C_{eq} is the equilibrium concentration of tracer gas in the air [PPM], E is the emission rate [PPM/h], λ is the ACR [h^{-1}], V_r is the room volume [m^3], t is time [h].

After solving equation (3) for E, the emission rate for each data point of the build-up measurement was calculated. The average value of the emission rates was converted from PPM/h to m^3/h using the following equation:

$$\dot{v} = V_r \cdot \frac{E}{10^6} \qquad (4)$$

$\dot{v}$ [m^3/h] is the air transfer from the crawl space to the living room at location C6, where an actual build up was measured (Paper I). Part of the aim for this PhD study was to investigate the possibility of conducting air transfer measurements only using equipment available for the decay method. Thus, instead of conducting a normal constant concentration measurement, the build-up of tracer gas was measured in the room while the tracer gas level in the crawl space or attic was significantly higher but not constant, which is a limitation of the method used in the study. The results of this calculation should therefore only be regarded as an estimation and attempt to use a simplified method for getting knowledge of the air transfer between construction cavity and living area. To more accurately calculate the air transfer from the cavity to the living area, it would have been necessary to connect a dosing device to the gas monitor, and thereby ensuring a constant concentration of tracer gas in the cavity. Another way would be to use a 2-zone model, e.g. the PFT method. However these methods would require more advanced equipment and a considerably longer measuring period, especially considering the small air changes in the cavities [73]. Also studies claim that a disadvantage of the PFT method is that it tend to overestimate the ACR due to adsorption on the surfaces in the room [74, 93]. In the case of the inaccessible construction cavities, the surface to room volume ratio is rather large, and the PFT method would therefore not be suitable, for either ACR measurements or air transfer measurements in the present study (Table 2-2).

3.1.3. STUDY DESIGN FOR INACCESSIBLE CAVITIES

For measurements in the inaccessible construction cavities, it was necessary to invent a design that allowed for proper mixing of the air in the cavity as well as for dosing and sampling of the air. This was done by a sealed circulation design, where dosing and sampling tubes were applied through holes drilled into the wall or floor structure, The mixing was ensured by connecting a leaf blower and a suction hose to holes drilled at opposite ends of the wall or floor surface. The leaf blower and suction hose was connected by the inlet of the leaf blower and the outlet of the leaf blower was connected to a hole in the wall or floor structure (Figure 3-1). A detailed description of the measurement design and mixing can be found in Paper I.

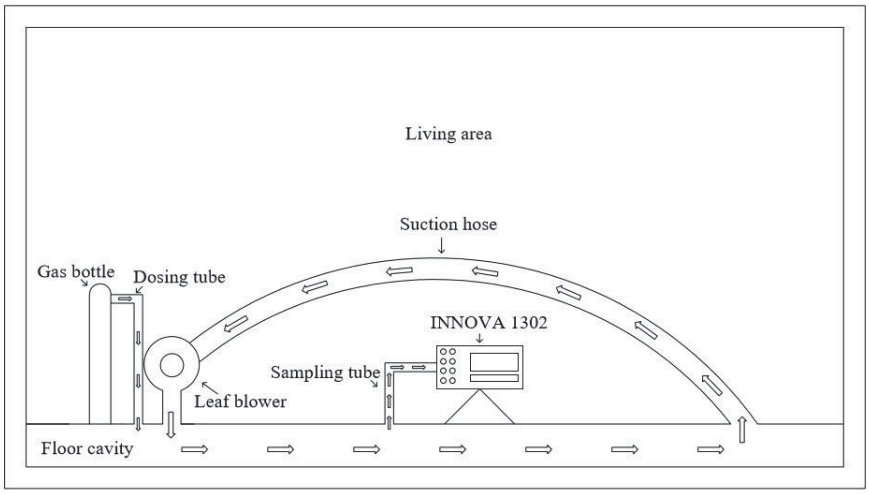

Figure 3-1. Example of the design for ACR measurements in the inaccessible cavities. The outlet of a leaf blower is connected to a hole in one end of the floor, and a suction hose is connected to both the inlet of the leaf blower and a hole in the opposite end of the floor. The decay of tracer gas is measured through a hole in the middle of the floor and the tracer gas is dosed into the cavity through a hole close to the leaf blower.

3.2. STUDY DESIGN FOR LOW CONCENTRATION FUNGAL SAMPLES (LABORATORY STUDIES)

Investigations of the release of fungal aerosols from infested gypsum boards, as well as the measurements of the TIP of the generated fungal samples, were conducted in a controlled environment of a laboratory. The fungal aerosol samples described in Paper II and III were generated from water-damaged gypsum boards inoculated with fungi sampled in a moisture-damaged house. A flow diagram of the release studies of fungal aerosols can be found in Figure 3-2. The investigations included six sampling scenarios (Table 3-2). A more detailed description of the incubation process of the gypsum boards, the sampling scenarios, and the general set up of the release study can be found in Paper II and III.

Further, fungal aerosol samples of *Penicillium brevicompactum* and *Aspergillus Fumaigatus* grown on Malt agar, and a surface scrap of *P. brevicompactum*, was generated as high concentration samples for test of the dose-response relationship between the TIP and fungal concentrations of a sample.

Table 3-2. Sampling scenarios (1-4) for the study of fungal particle release, and surfaces sampling scenarios (5-6), from infested gypsum boards in Paper II.

Sampling scenarios	Sampling type	Repetitions	Sampling time	Sampling flow	Incubation RH
1	Impinger GSP APS	3	15 min	9.0 m/s	Damp (>95 %)
2	Impinger GSP APS	3	8 h	2.5 m/s	Damp (>95 %)
3	Impinger GSP APS	3	15 min	9.0 m/s	Dry (35-40 %)
4	Impinger GSP APS	3	8 h	2.5 m/s	Dry (35-40 %)
5	Scrapings Imprint plate	6	-	-	Damp (>95 %)
6	Scrapings Imprint plate	6	-	-	Dry (35-40 %)

Impinger = BioSampler SKC, GSP = GesamtStaubProbenahme, APS = Aerodynamic Particle Sizer.

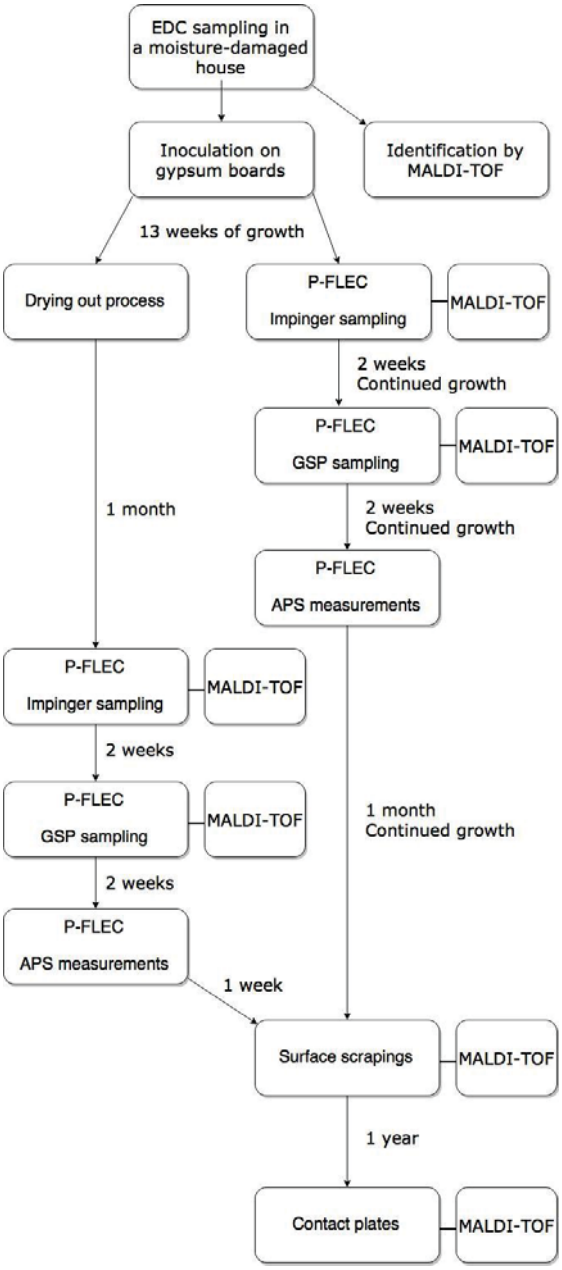

Figure 3-2. Flow diagram of the sampling and incubation process used for the release study in Paper II. EDC = Electrostatic Dust Collector, MALDI-TOF = Matrix-Assisted Laser Desorption/Ionization-Time-Of-Flight, P-FLEC = Particle-Field and Laboratory Emission Cell, GSP = GesamtStaubProbenahme, impinger = Biosampler SKC, and APS = Aerodynamic Particle Sizer.

3.2.1. GENERATION OF FUNGAL AEROSOLS

To generate the fungal aerosols, the Particle-Field and Laboratory emission Cell (P-FLEC) was used as described by Kildesø et. al [23, 94]. The P-FLEC is a cylindrical cup-shaped device. Inside the chamber, a bar with ten 0.8 µm nozzles are rotating 1 cm above the surface, at a speed of 60 s per rotation. Through the nozzles in the bar, small air jets are directed towards the surface in an angel of 45 ° and scan a surface of 130 cm^2. The aerosols released from the surfaces, are transported to the outlet at the top of the P-FLEC by the airflow created in the chamber [95] (Figure 3-3). Sampling and measuring devices can be connected to the outlet at the top. For the present PhD study, the Aerodynamic Particle Sizer (APS), the GesamtStaubProbenahme (GSP) (CIS by BGI, INC, USA) and the BioSampler (impinger) (SKC, UK) were used.

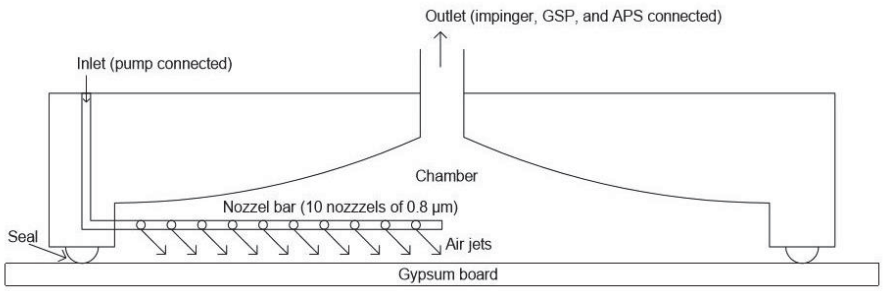

Figure 3-3. A cross sectional view of the P-FLEC placed on a gypsum board. The nozzle bar is placed 1 cm above the surfaces of the gypsum board, and the through the nozzles, air jests are directed towards the surface at an angle of 45 °.

3.2.2. QUANTIFICATION OF PARTICLES

The APS was used for quantification of released particles in Paper II. The APS was connected to the outlet at the top of the P-FLEC and measured the aerosolised particles every second in 51 size ranges between 0.54 µm and 19.8 µm. The APS has in previous studies been used to measure the aerodynamic diameter and size distribution of fungal aerosols in laboratory settings [96, 97] and in environmental studies [98, 99].

The geometrical mean (GM) uses the product of the values and thereby present the typical value or central tendency of the particle sizes in the data set, making them comparable in a more manageable way. Thus, to compare the particle data measured with the APS in Paper II, the geometrical mean diameter (D_g) was calculated using the following equation:

$$D_g = (D_1{}^{n_1} \cdot D_2{}^{n_2} \cdot D_3{}^{n_3} \dots D_n{}^{n_n})^{1/N} \qquad (5)$$

D_g is the geometrical mean diameter, D_1 is the geometrical midpoint of the interval, n_1 is the measured number of particles in the interval, and N is the total number of particles summed over each interval.

3.2.3. IMPINGMENT AND FILTER SAMPLING

Impingement is a sampling technique, where aerosols are sampled into a sampling liquid. In the present study the new generation impinger, BioSampler, was used. The BioSampler has in previous studies shown to be more efficient compared to the AGI 30 impinger [100, 101], especially in regards to long-term sampling (above 30 min). However, during long-term sampling, evaporation can be a concern and refilling of the sampling liquid might be necessary [100, 101]. Liquid impingement has been suggested to also collect airborne mycotoxins [102, 103], which would be of interest in regards to the investigation of the TIP of the fungal samples. In previous studies, impingement have been used for sampling of airborne microorganism [48, 104] and have shown convincing results [105]. The three nozzles in the BioSampler are located just above the sampling liquid (Figure 3-4) and create a swirling movement in the liquid which gently collect the aerosols into the sampling liquid and minimizes re-aerosolisation [100, 106]. The impinger samples aerosols at a flow of 12.5 l/min into 20 ml of sampling liquid; in this study a 0.0005% Tween20 solution was used (Paper III).

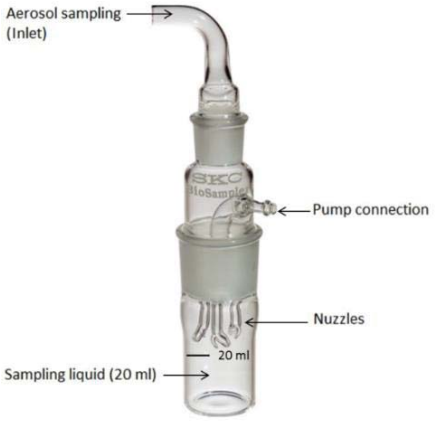

Figure 3-4. BioSampler (impinger)(SKC, UK) used for liquid sampling of fungal aerosols.

The GSP is a filter sampling system, and the one used in the present PhD study samples at a flow of 3.5 l/min. The GSP sampler is used for sampling of inhalable aerosols [107], thus often used in studies to sample airborne dust [56, 108, 109] or in studies of aerosols released from fungal infested building materials [53]. A polycarbonate filter with pore size 1 μm (Frisenette, USA) was installed in the GSP sampler cassette to capture the fungal aerosols released from the gypsum boards. The fungal particles on the filters were after sampling extracted in 5.6 ml 0.0005% Tween20 solution (similar to the impingement liquid) by shaking at 300 RPM for one hour (Paper II and III).

3.2.4. IDENTIFICATION BY MALDI-TOF

The MALDI-TOF MS (Matrix-Assisted Laser Desorption/Ionization-Time-Of-Flight Mass Spectrometry) has in recent years been described as fast, accurate, and easy to use for identification of fungi and bacteria on species level [110]. The MALDI-TOF techniques has been used in previous studies for identification of microbes in environmental samples [52, 111], and in laboratory generated samples from infested building materials [52]. Thus, the MALDI-TOF technique was suitable for identification of fungi and bacteria in the present PhD study.

The species identification by MALDI-TOF MS is performed by applying a matrix to a fungal- or bacterial sample on a steel target plate. When the matrix crystalizes upon drying, the fungal- or bacterial sample underneath it likewise crystalize. A laser beam shoot at each sample on the target plate, and the sample ionizes. The time of flight for the ions is measured and used to determine the peptide and protein mass fingerprint (PMF) which is then compared to the PMF's in the database, to identify the fungal- or bacterial species of the sample on the target plate. Thus one of the main limitations of the technique is that the fungal- or bacterial species can only be determined if its PMF is available in the database [110]. For analysis, an Autoflex III (Bruker Daltonics, Germany), using the Bruker Biotyper 3.1 software with the BDAL filamentous library 1.0, was used. A detailed description of the identification procedure can be found in Paper II and III.

3.2.4.1 ERMI index and value

The ERMI index is a fungal index developed in the US, grouping common indoor fungal species in two groups. Group 1 are typically associated with fungal growth or dampness in buildings, and group 2 are often found in buildings, but not associated with fungal growth or dampness. The index consists of 36 fungal species, or groups of species, where 26 belong to group 1 and ten to group 2. The 36 fugal species, or groups of species, were selected out of a set of 82 fungal species, that were widely distributed across the US [40]. Geographical differences may occur in dividing the fungal species in the two groups, or when determining which species belong in the

index. This should be borne in mind as a limitation of the index [46]. The fungal species in the ERMI Index have been identified by use of PCR analysis.

An ERMI value can be calculated as a measure for the fungal burden of the building. When the concentration of each fungal species in a sample is known, the ERMI value can be calculated by the following equation [40]:

$$ERMI\ value = \sum_{i=1}^{26} \log_{10}(s_{1i}) - \sum_{j=1}^{10} \log_{10}(s_{2j}) \quad (6)$$

S_1 is the sum of $\log_{10}$ of the concentrations of group 1 fungi measured in the homes, and S_2 is the sum of $\log_{10}$ of the concentrations of group 2 fungi measured in the homes.

In Paper II, fungal species in dust samples from five non-moisture damaged Danish homes were identified by use of the MALDI TOF MS technique, and following analysed against the ERMI index to identify parameters that might influence the fungal burden of the homes, such as season, ACR, and RH. The MALDI-TOF MS database used for identification included 27 of the 36 species in the ERMI index. The calculated ERMI values are referred to as M-ERMI values since the species identification has been made by MALDI-TOF MS analysis.

3.3. INVESTIGATION OF THE INFLAMMATORY POTENTIAL

The TIP of the fungal samples was investigated by use of the granulocyte assay, which is based on differentiated HL-60 cells (Human Promyelocytic Leukaemia cell line). The granulocyte assay is determining the inflammatory potential of a sample by measuring the reactive oxygen species (ROS). The granulocyte assay has in previous studies proven to be sensitive to microorganism [53, 81, 112, 113], in fact has a study shown that it has a lower detection level of the inflammation caused by yeast compared to two alternative assays [113]. Further, the granulocyte assay is suitable for measurements of the inflammation caused by multiple microorganism and is a fast way to determine the inflammatory potential of a sample compared to other available methods [113], which ensures that the fungi in the samples, do not multiply during the analysing period. Thus, the granulocyte assay is suitable for investigation of inflammatory potential of the fungal samples in the present PhD study.

The HL-60 cell line was established from a Croatian female patient with acute leukaemia in 1977, and upon exposure to fungi, the differentiated HL-60 cells will react by producing ROS. The ROS emitted by the cells will react with luminol, which is added to the cell suspension along with human plasma and the fungal suspension. Through the reaction with luminol, light will be emitted, which can be detected by a thermostated (37°C) ORION II Microplate luminometer (Berthold Detection Systems, Germany). The luminometer measures the relative-light units per second (RLU/s) every second for a period of three hours [113]. All samples were conducted

in duplicates, the RLU/s values were added, and an average value of the duplicate was calculated representing the TIP of each sample. A detailed description of the procedure can be found in Paper III.

3.4. VIABILITY TEST OF HL60 CELLS

Different assays to test for cytotoxicity of a sample are available [114], one way is to conduct a viable and dead cell count. A study has found that viable and dead cell count on a NucleoCounter NC-100^{TM}(Chemometec, Denmark) show similar results as to an assay measuring lactate dehydrogenase release [115]. Other studies using different chemical agent, have likewise used the NucleoCounter for viability test of mammalian cells in cytotoxicity studies [116, 117]. In the present PhD study the viability of the granulocyte like cells during exposure to the fungal samples, was of interest. Thus, using the NucleoCounter to conduct viable and dead cell count of the suspensions, seemed a suitable solution. The viable and dead cell count was done after 40 min and 90 min of exposing the granulocyte like cells to the fungal samples. Prior to the cell count, it was tested if the NucleoCounter would confuse the fungal particles in the suspension with granulocyte like cells - this was not the case.

3.5. STATISTICAL ANALYSES

The static analyses preformed in the present PhD study can be found in Table 3-3. All static analyses were made using the Static Analysis Software (SAS 9.4). All values were log transformed for comparison.

Table 3-3. Static analyses preformed in the PhD study. All values were log transformed for comparison and all analyses were carried out in the Static Analysis Software (SAS 9.4).

Paper II	**Paper III**	**Analysis**
Investigation of the influence of dampness and air velocity in combination with sampling time on the release rate and total release of fungal aerosols from gypsum boards.	Investigation of the influence of dampness, air velocity in combination with sampling time, and sampling type on both TIP and the total concentration of the aerosol samples.	*Anova*
Comparison of the M-ERMI values as affected by season, with random effect of homes.	Comparison of the concentration of each fungal species in the sample.	*Mixed*
Investigation of the influence of RH and ACR on both M-ERMI and total fungal concentrations of samples from five non-moisture damaged homes.	Investigation of the correlation between the concentration and TIP of the fungal samples, between the TIP of the undiluted and 10X diluted samples, and between the concentrations of *Aspergillus versicolor* and *Penicillium chrysogenum.*	*Correlation*
Not performed	Comparison of differences between data point on the dose-response curves.	*Paired T-test*

CHAPTER 4. DISCUSSION OF MAIN FINDINGS

In this chapter, the main findings from Paper I-III are summarise and discussed.

4.1. ACR IN BUILDINGS

The measured ACR of the living areas (Paper I and Table 4-1), were in general below the recommend level of the Danish building code (equivalent to 0.5 h^{-1}, cf. Chapter 2.2) [62]. Likewise has ACR's below the recommended level of the Swedish building code (equivalent to 0.5 h^{-1}) been found in a study of 390 Swedish homes [118]. When constant mixing was applied during the measurements, the ACR in both cavities and living areas increased by 0.03-0.16 h^{-1} (Table 4-1). This is in accordance with a previous study also using the tracer gas decay method, which likewise found small increases in the ACR when using constant mixing [119]. Even if the increase in ACR in the present PhD study was rather small, it should be borne in mind that use of constant mixing during ACR measurements might overestimate the measured ACR. The crawl space at location C1 (Table 4-1), was the only exception to this. Here the ventilation rate decreased during constant mixing. This could perhaps be explained by the observed changes in weather conditions. The measurements of ACR in the crawl space at location C1 were carried out over a two-day period, where the weather conditions changed significantly, by increase of both temperature and wind speed between the first measurement (with constant mixing) and second measurement (without constant mixing). Studies have shown that the outside weather conditions influences the ACR of the buildings [14, 73].

The measured ACR in the wall cavity at the office building (W1), with mechanical exhaust, showed that an increase in ACR of 0.33 h^{-1} occurred in the wall cavity if the mechanical exhaust in the two adjacent rooms was operating. When the mechanical exhaust in a room is operating, it may be assumed that an increase in ACR in the room likewise will occur. This indicates that the ACR in the adjacent room might influence the air transfer from the cavity to the indoor air. In the present study mechanical exhaust was applied in both rooms surrounding the wall structure, and they were operating at the same time. It would however be of interest to investigate if mechanical exhaust in only one of the adjacent rooms would result in an increase of air transfer through that room, compared to adjacent rooms with no mechanical exhaust. However, to my knowledge no such studies have been conducted. The present PhD study is not an in-depth investigation on the influence of ACR on the air transfer between locations. Thus to decisively conclude on this matter, further studies using different measurement techniques and multiple comparable locations, should be considered - as also suggested in a review by WHO [14].

Table 4-1. Results of the air change rate measurements with indications of constant mixing.

Locations	Constant mixing	Air Change [h^{-1}]
A1		
Adjacent living space	+	0.28
Attic ventilated	+	7.6
Attic reduced ventilation	+	3.29
A2		
Adjacent living space	+	0.36
Attic ventilated	+	43.2
W1		
Adjacent room	+	0.23
Partition wall mechanical exhaust on	-	0.97
Partition wall mechanical exhaust off	-	0.64
W2		
Adjacent living space	+	0.12
Partition wall	-	0.19
F1		
Adjacent living space	+	0.1
Floor cavity	-	0.05
C1		
Adjacent living space	+	0.20
Adjacent living space	-	0.13
Crawl space	+	0.28
Crawl space	-	0.78
Crawl space without ventilation	+	0.71
Crawl space without ventilation	-	0.33
C2		
Adjacent living space	+	0.34
Adjacent living space	-	0.25
Crawl space	+	0.72
Crawl space	-	0.56
Crawl space without ventilation	+	0.55
Crawl space without ventilation	-	0.52
C3		
Adjacent living space	+	0.77
Crawl space	+	1.19
C4		
Adjacent living space	+	0.86
Crawl space	+	2.27
C5		
Adjacent living space	+	0.49
Crawl space	+	0.77
C6		
Adjacent living space	+	1.44
Crawl space	+	1.45
C7		
Adjacent living space	+	0.28
Crawl space	+	0.77

4.1.1. AIR TRANSFER FROM CAVITY TO LIVING AREA

The air transfer from cavity to living space was measured in a total of four locations, A1, A2, C6 and C7. For the attics (A1 and A2), the tracer gas measured in the living areas was at background level (< 1 PPM of SF_6) and for the crawl space at C7, no build-up of tracer gas was measured, but the level of tracer gas in the living area was slightly above the background level (≈2.7 PPM of SF_6) (Paper I). However, at location C6, a build-up of tracer gas was measured (Figure 4-1). Thus for location C6, it seemed possible to estimate an air transfer of 8.24 m^3/h, using equation 3 and 4 (Paper I). However, it should be borne in mind that the lack of a constant tracer gas concentration in the crawl space will unavoidably present uncertainties in the calculation of the air transfer as described in Chapter 3.1.2. Despite this, the results indicate that the tracer gas is penetrating the floor structure at location C7 but at a significantly lower rate than at location C6. A reason for this could be that the floor structure at location C7 was significantly better insulated and tighter than the floor structure at location C6, where actual cracks in the structure was observed (Paper I).

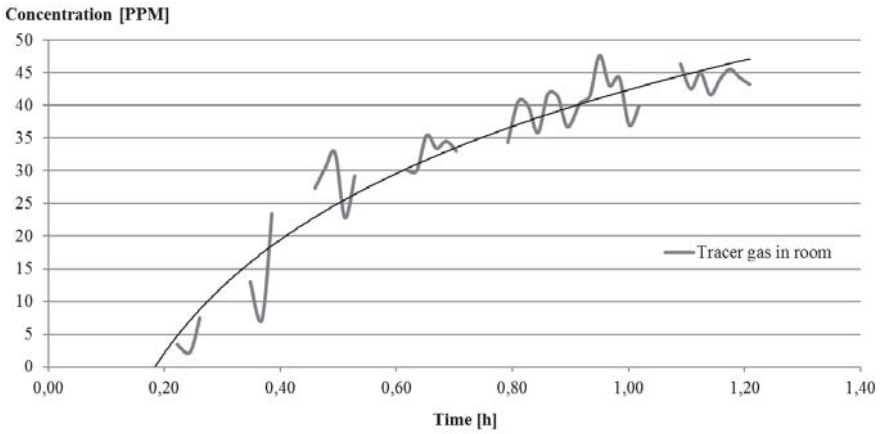

Figure 4-1 (Paper I). The measured build-up of tracer gas in the living space at location C6. The tracer gas level in the crawl space was kept high, however not constant.

The study of the air transfer indicates that crawl spaces contribute more to the pollution of the indoor air than the attics (Paper I). A reason for this could be that perhaps the air transfer from attics to living areas underneath might be affected by season. That air from crawl spaces is affecting the indoor air in the living spaces above has also been suggested in previous studies. E.g. did a Danish study using tracer gas measurements, find that up to 95% of the crawl space air travels up into the living area above [120]. Further, a Finish study investigating fungal contamination of the indoor air from crawl spaces, found that air leakage from crawl spaces to living areas occurs [31]. Unfortunately, to my knowledge no studies comparing differences in exposure risk from different construction cavities (e.g. crawl spaces and attics) have

been conducted, thus weakens the conclusion on this matter. However, literature point towards such investigations being of importance [121-123]. E.g. did a study find that the transport of fungal spores through a building structure not only depend on the pressure difference between outside and indoors, which is influenced by the ACR of the building [124], but also on the fungal particle size [125], making interdisciplinary studies of fungal infested buildings highly relevant. On the other hand, a study conducted in a subarctic climate did not find that fungal growth in an external wall contributes to the contamination of the indoor air, but still concludes that microbial contamination from the building envelope should not be ignored. Together, this points towards three factor being of particular interest when evaluating the exposure risk of the indoor air from construction cavities. (1) The tightness of the separating structure, (2) the airborne fungal species composition of the cavity air, and (3) the type of cavity - e.g. if it is a crawl space or an attic.

4.2. AIRBORNE FUNGI IN BUILDINGS

The calculated M-ERMI values of the five non-moisture damaged homes divided by season, the total concentration of measured airborne fungi in the homes over the four seasons, and the geometrical mean value of the ACR and RH for each home can be found in Table 4-2. The ACR and RH are obtained from a previous study including the same homes [56] (Paper II).

Table 4-2 (Paper II). The total concentration of airborne fungi for each home, the M-ERMI values for each season, the geometrical mean value of ACR and RH for each home.

Homes	Total concentration [CFU/m^2/day]	M-ERMI value Summer	M-ERMI value Autumn	M-ERMI value Winter	M-ERMI value Spring	ACR over season [h^{-1}] [56]	RH over season [%]
A	14,035	-3.71	4.88	4.40	0	0.4	48
B	17,544	-0.90	13.01	5.69	-6.60	0.8	55
C	16,746	-3.20	4.88	11.31	4.71	0.2	56
D	15,630	-4.45	12.39	5.18	7.39	0.2	53
E	18,979	-3.61	6.90	-	0.30	1.5	56
Estimate	-	-9.8227	1.7683	-	-4.2898	-	-
P-value	-	0.0003	0.42		0.062	-	-

The ACR of the homes correlated negatively with the M-ERMI value of the homes ($r=-0.689$, $P=0.0011$, $n=19$), indicating that the ACR has a beneficial effect on the species composition of the indoor air. Further, the M-ERMI values were significantly associated with season ($P=0.0002$, $n=19$), so that the M-ERMI values were higher in winter compared to summer and spring (Table 4-2). However, the highest concentration of airborne fungi in the five homes was found during summer ($P<0.0001$, $n=19$) (Table 4-3), and the ACR of the homes and the total concentration of airborne fungi correlated positively ($r=0.667$, $P=0.0018$, $n=19$). In summer, typical outdoor species (e.g. *Cladosporium* sp.) were found more frequently in the indoor air compared to winter (Table 4-3). Together, this indicates that the outdoor air was the

dominating source of indoor airborne fungi in the studied homes - especially during summer. This is in accordance with a previous study which likewise found indications that the outdoor air is the dominating source of airborne fungi in the indoor air in summer time [56]. Other studies have also found that the outdoor concentration of fungi influences the indoor concentration [60, 126, 127]. This is likely due to infiltration and ventilation of buildings, where outside air is replacing the indoor air and thereby caring the fungal particles inside. This can be minimized by installation of mechanical ventilation where filters remove a large percentage of fungal particles before the air is sent into the building. However, the ventilation system must be properly installed and maintained [14].

The M-ERMI values varied between the homes (Table 4-2), and a positive association was found between the RH and the M-ERMI value of the homes ($r=0.527$, $P=0.024$, $n=18$). This indicates that either user behaviour or individual building characteristics might affect the exposure to airborne fungi in the indoor air of the homes. This is in agreement with a study investigating the effect of building characteristics and occupants behaviour on the moisture levels of 105 Danish homes [1]. The study found that user behaviour, such as drying clothes, contributed to the RH of the homes. Several other studies [4, 5, 84, 128-130], including reviews [7, 14, 18], have in addition found a positive association between elevated moisture levels and concentration of airborne fungi in buildings. The positive association between RH and M-ERMI values also indicate that a high moisture accumulation of the indoor air will have a negative effect on the species composition, since a higher ratio of fungi associated with mouldy buildings will be present.

A stepwise regression analysis including ACR, RH, and season, showed a significant association between M-ERMI values and ACR (Estimate=-6.08, $p=0.0005$) and RH (Estimate=32.96, $p=0.007$) (Paper II). Pointing towards ventilation and moisture control of a building as the most important parameters to influence the exposure risk to fungal aerosols, as review studies have likewise concluded [7, 14].

Table 4-3. Identified species of the five non-moisture damaged homes, divided by season. The numbers of homes with positive samples are indicated for each species and season.

	Summer	Autumn	Winter	Spring
	Number of homes positive for the species			
Aspergillus				
A. flavus[a]		1		
A. fumigatus[a]	1			
A. glaucus		1		
A. niger[a]				1
A. nidulans	2	1		1
A. penicilloides[a]		1		
A. versicolor[a]		1	2	1
Candida				
Ca. lambica[a]	1			
Ca. sorbosa[a]	1	1		
Chaetomium				
Ch. globosum[a]		1		
Cladosporium				
C. cladosporides[b]	4			1
C. herbarum[b]	5	1		1
Epicoccum				
E. nigrum[b]	1			
Fusarium				
F. proliferatum				1
Penicillium				
P. brevicompactum[a]	4	5	3	2
P. camemberti	1	1	3	1
P. chrysogenum[b]			1	1
P. citrinum			1	
P. corylophilum[a]		1	1	
P. glabrum[a]		5	3	2
P. olsonii		2		
P. roqueforti				2
Wallemia				
W. sebi[a]		2		1
Total concentration	**12,177**	**1,430**	**518**	**626**

[a] Fungi frequently found in US homes and associated with mouldy buildings (Group 1).
[b] Fungi frequently found in US homes but not associated with mouldy buildings (Group 2).
Total concentration is in CFU/m^2/day, the values are the geometrical mean of the homes.

4.3. SPECIES COMPOSITION IN GYPSUM BOARD SAMPLES

A detailed overview of the species composition for each aerosol- and surface sample can be found in Paper II and III. A total of 17 fungal species were recovered in the EDC sampling in the moisture-damaged house from Paper II (Table 4-4). Four of the 17 fungal species were recovered in the aerosols sampled by GSP, three of the 17 fungal species were recovered in the aerosols sampled by impinger (Paper III), and five of the 17 fungal species were recovered in the surfaces scrapings (Table 4-4). Four of the 17 fungal species were recovered on the imprint plates taken on the previously damp surfaces whereas only two of the 17 fungal species were recovered on the imprint plates taken on the dried surfaces (Table 4-4). Further, *Stachybotrys chartarum* was only found on the surfaces of the damp gypsum boards by visual inspection and microscopy (therefore not indicated in Table 4-4). That *S. chartarum* was only found on the damp surfaces, is in accordance with a previous study showing that *S. chartarum* need elevated material moisture levels to proliferate on buildings materials [131].

Table 4-4. Identified species in samplings from gypsum boards and the moisture-damaged building (inoculum) in Paper II and III. EDC was used for sampling of fungi in the moisture-damaged building.

Species	**Inoculum n=2**	**GSP n=12**	**Impinger n=12**	**Scraping n=12**	**Imprint n=12**
Aspergillus flavus	+	+[c]			
A. fumigatus	+	+[b]			
A. niger			+[c]		+[b]
A. nidulans					+[b]
A. penicilloides					
A. ustus	+			+[b]	+[b]
A. versicolor	+	+[bc]	+[bc]	+[bc]	
Botrytis cinerea	+				
Chaetomium globosum	+			+[c]	
Cladosporium cladosporides	+				
C. herbarum	+				
Penicillium dierckxii	+				
P. brevicompactum	+				
P. chrysogenum	+	+[bc]	+[bc]	+[bc]	+[bc]
P. commune	+				
P. corylophilum	+				
P. glabrum	+			+[c]	
P. olsonii	+				
P. verrucosum	+				+[c]
Stachybotrys chartarum	+				
Bacillus infantis[a]				+	
Paenibacillus sp[a]				+	

[a] Bacteria, [b] Recovered in samples from the damp surfaces, [c] Recovered in samples from the dry surfaces, and n=number of samplings.

The difference in species richness between imprint plates from previously damp and dried surfaces, indicates that the species richness on a fungal infested surface, and thereby species composition of the indoor air, is influenced by drying of infested surfaces. This is supported by a previous study which found that fungal growth on ceiling tiles could be limited if the surfaces was died fast and kept dry [132]. That the dampness (a_w) of the infested surface has an influence on the species composition is observed in research studies and literature reviews [17, 18, 22, 133], where it is concluded that the fungal species have different requirements to a_w of the surfaces in order to proliferate. It is indisputable that the difference in growth on the surfaces also affects the composition of the measured airborne fungal species. In the present PhD study, different species were recovered in the aerosols from damp and dry surfaces (Paper III and Table 4-4).

Some species were only found in the surface samples and not recovered in the aerosol samples. This indicates that the release of fungal spores to indoor air depends on the individual species. This is in agreements with previous studies, which have found that the aerosolisation of fungi, amongst other things, depends on the shape of the spores and the length of the spore chains [134, 135]. Further, *Chaetomium globosum* was only found on the dry surfaces (Table 4-4) and not recovered in any of the aerosol samples. This might be explained by a study that investigated aerosolisation of *Ch. globosum* spores, and found that its spores grow in slime heads, making them less likely to become airborne [136]. Also, only a subsample of each spore suspension was used for identification and thus only dominating species were identified.

In general *Aspergillus versicolor* and *Penicillium chrysogenum* were the two dominating species in both aerosol samples and surfaces samples, which is in agreement with previous studies investigating fungal growth on building materials. E.g. did studies find that *A. versicolor* and *P. chrysogenum* were by far the most frequently found species on damp building materials [19, 137], and that *P. chrysogenum* grows rapidly on gypsum boards [137]. Further, a study found *Penicillium* sp. to be released more frequently from gypsum boards compared to other building materials, such as mineral insulation, wood, and ceramic products [28].

The fungal samples were tested for presence of bacteria (Paper III). Only two bacterial species were found, *Bacillus infantis* and *Paenibacillus* sp. and they were only found in the surface samples and not in the aerosol samples (Table 4-4). This indicates that bacteria are present as part of fungal infestations in buildings. This is in accordance with a previous study [28], but the present PhD study adds that the bacteria are not easily aerosolised. Studies have found levels of airborne bacteria in the indoor air to be negatively associated with ACR [5, 56] and positively associated with floor area per person [5] or presence of occupants [82], and several studies have showed that bacteria in an aerosol sample contributes to the inflammatory potential of the sample [56, 81, 83, 138]. Thus, the presence of bacteria on fungal infested surfaces in buildings should be considered during a remediation process.

4.3.1. INFLUENCE OF SAMPLING TIME AND SAMPLING TYPE

Aspergillus fumigatus and *Aspergillus flavus*, were only found in the long-term samplings (Paper III). This could simply be due to differences in growth on the surfaces, since the long- and short term samplings were not conducted on gypsum boards incubated in the same boxes. However, it is also likely that the species composition of an aerosol sample might be affected by the sampling time, since some fungal species become easier airborne due to their physiology (cf. Chapter 4.3). However, to my knowledge, no previous aerosolisation studies have been done with long sampling times as the present study with 8 h, which weakens the conclusion on this matter.

In the present PhD study, a difference was seen in recovery of fungal species between the GSP sampling and impingement, even if the gypsum boards were incubated in the same boxes and therefore may be assumed to have similar fungal growth (Paper II and III). Only *Aspergillus versicolor* and *Penicilium chrysogenum* were recovered in both GSP and impinger samples (Table 4-4). Further, there was a tendency towards the impinger (GM=$2.8X10^5$ CFU/m^2) sampling a larger amount of fungi than the GSP (GM=$6.6X10^4$ CFU/m^2) in connection with the damp surfaces (p=0.14). However, the GSP (GM=$5.6X10^4$ CFU/m^2) tended to sample a larger amount of fungi than the impinger (GM=$1.7X10^4$ CFU/m^2) in connection with dry surfaces (p=0.30) (Paper III). Together, this indicates that some viable fungal parts lose their viability in the dry environment of the GSP sampler. This hypothesis is strengthened by a previous study showing that sensitive microorganism, such as *Legionella*, was better recovered by liquid impingement [139]. The fungal spores surviving the sampling by the GSP might be spores which have developed a protective layer of melanin [140, 141] during the drying process. The layer may have made the spores more robust, and thus their survival is not affected by the sampling time on dry filters in the GSP.

4.4. RELEASE FROM INFESTED SURFACES

The highest fungal particle release rate (p=0.0003, n=10) was found from damp surfaces, exposed to high air velocity of 9 m/s. The same was the case for the total release of fungal spores (p=0.0025, n=10) - despite a shorter sampling time of 15 min versus 8 h (Figure 4-2). For the total particle release from dry surfaces the opposite was observed (p=0.0025, n=10) (Figure 4-2). This indicates that for damp surfaces with substantial growth, the air velocity near the surfaces, greatly affects the release and thereby exposure to airborne fungal particles. However, for the dry surfaces with less substantial growth, the sampling time seems to be of importance. The importance of sampling time for the dry surfaces could be due to the species growing on the surfaces - e.g. *Ch. globosum* (c.f. Chapter 4.3).

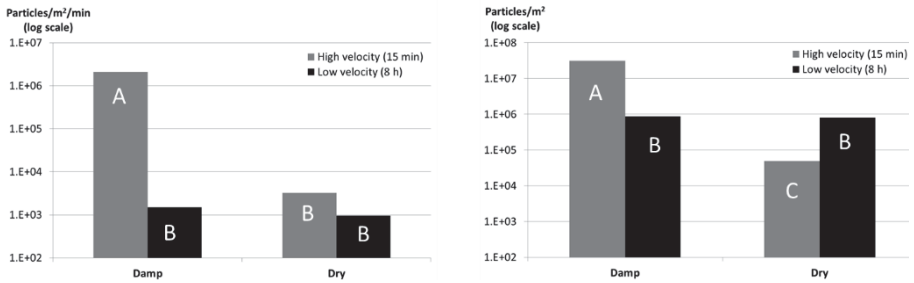

Figure 4-2. Left, the fungal particle release rate, and right, the total fungal particle release. There is no statistical difference between columns with the same letter.

A previous study of fungal growth on gypsum boards, found that dry surfaces contributed more to the concentration of airborne fungi compared to damp surfaces [53]. The same results were found in a study using agar as growth medium [36]. In Paper II, the damp surfaces released more fungal particles compared with the dry surfaces, a reason for this might be the design of the study, where the dry surfaces were dried out before gypsum boards where overgrown. Further, the gypsum boards studied in Paper II were exposed to a larger air velocity and a considerable longer sampling time, which are expected to cause a higher release and release rate from surfaces with more substantial growth. Thus, the results of the release of fungal particles to the indoor air indicate that, at high air velocities near the surface, immediate action in the remediation process of fungal infested buildings seem important to reduce the exposure risk for the occupants.

When investigating the particle release over time (Figure 4-3), the same tendency was seen for all measurements, where the release rate decreased as time passed. However, after 8 hours of sampling with a low velocity, fungal particles still became airborne. This indicates that the sampling time might affect the measurements of particle release rate from infested surfaces.

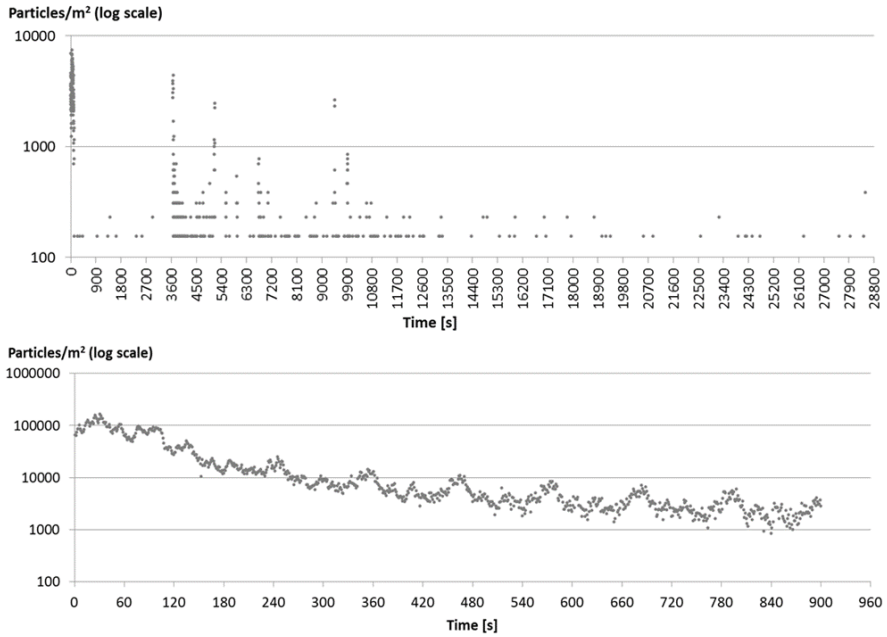

Figure 4-3 (Paper II). Examples of decrease in fungal particle release rate over time. Top shows an example of the release of fungal particle during a long-term sampling with low velocity over a dry surface. Bottom, shows an example of the release of fungal particles during a short-term sampling with high velocity over a damp surface.

4.5. INFLAMMATORY POTENTIAL

The fungal samples used for analysis of the inflammatory potential (Paper III), contained rather low fungal concentrations (10^2-10^5 CFU/ml; GM=$1.43*10^4$), which is in the same range as studies using environmental samples [56, 82]. These studies often contain more than 100 times lower concentrations than fungal samples generated in the laboratory (10^5-10^7) [53, 81, 83]. The samples with low concentration of fungi were generated to test if even low levels of airborne fungi, e.g. caused by hidden fungal growth, might still trigger a response from the granulocyte like cells in the assay. However, an interesting phenomenon was observed, since the TIP of the 10X diluted samples where significantly higher (p=0.0005) than the TIP of the undiluted samples (Figure 4-4) and a positive correlation was found for the TIP of the diluted and undiluted samples (r=0.651, p=0.0005, n=24).

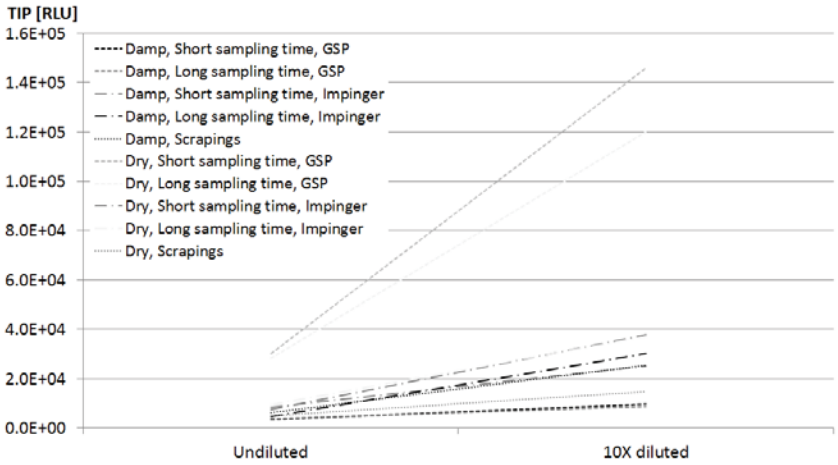

Figure 4-4 (Paper III). Graphic illustration of the relationship between the TIP of the undiluted and 10X diluted fungal samples. The illustration is not showing a development of TIP, thus the strait line in between the point are only to illustrate that the undiluted samples has a lower TIP compared to the diluted samples.

To test if the lower TIP of the undiluted samples was not in fact due to cytotoxicity of the samples, a viable cell count of the granulocyte like cells was preformed after 40 min and 90 min of exposure of the cells to a number of selected fungal samples. After 40 min of exposure no signs of cytotoxicity was observed for the granulocyte like cells (Figure 4-5). The same result was seen after 90 min of exposure (2-12 % cell death). Thus the fungal samples seem not to be toxic during exposure time in the granulocyte assay. It should however be born in mind that cell death due to cytotoxicity of a sample, might only occur after 24 hours of exposure, thus this test cannot be regarded as a general toxicity test of the fungal samples in the study. It is merely a check to test the viability of the granulocyte-like cells, during the exposure period of the granulocyte assay (Paper III).

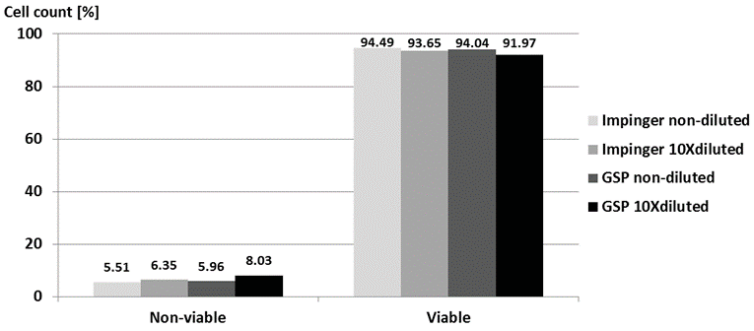

Figure 4-5 (Paper III). Percentage of non-viable and viable granulocyte like cells after 40 minutes of fungal exposure.

This lead to investigations of the dose-response relationship between the TIP of fungal samples and the fungal concentration in the samples. High concentration fungal samples of *Penicillium brevicompactum* (aerosol sample=$8.0X10^4$ spores/ml and surfaces sample=$1.5X10^8$ spores/ml) and *Aspergillus fumigatus* (aerosol sample=$2.1X10^6$ spores/ml), was used in the dose-response test. This revealed a tendency to a J-shaped dose-response curve (Figure 4-6). A paired T-test showed that there was no statistical difference between the two lowest concentrations (p=0.15) only between the two highest (p=0.02). Still the tendency was clear for all three tested fungal samples (Paper III). A non-linear dose-response curve has likewise been found in many both *in vivo* and *in vitro* toxicity studies, where different toxic agents, such as alcohol and a wide range of chemical agents, e.g. PCB, showed U-shaped dose-response curves [142-147].

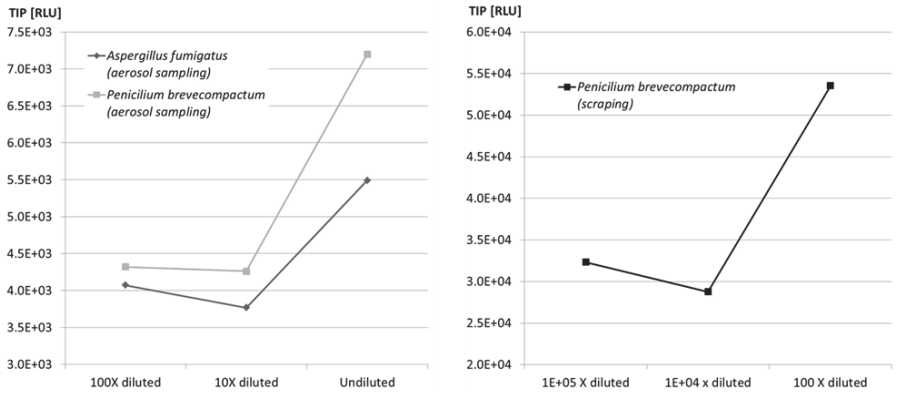

Figure 4-6. Doses-response curve for Penicillium brevicompactum and Aspergillus fumigatus cultivated on Malt extract agar. Left diagram shows the dose-response curve of aerosols from respectively A. fumigatus (undiluted $2.1*10^6$ spores/ml) and P. bervicompactum (undiluted $8.0*10^4$ spores/ml). Right diagram shows dose-response curve of scrapings from P. brevicompactum ($1.5*10^8$ spores/ml).

It seems that the fungal samples used in the granulocyte assay might only range in first part of the J-shaped dose-response curve, resulting in the diluted samples having a higher TIP than the undiluted samples (Paper III). The TIP of the undiluted samples from the gypsum boards samplings, was above the detection level for a few of the samples (Paper III), and thus only the diluted samples were used for analyses of the TIP in regards to dampness, sampling time, sampling type and fungal concentration. The results showed that there was no association between the fungal concentration and the TIP of the samples (r=0.137, p=0.42, n=24). This is in accordance with other studies using low concentration fungal samples [56, 82]. However, in studies with higher concentrations of fungal samples an association between the concentration and the TIP of the samples has been found [53, 81]. This might again be due to the non-linear dose-response curve, or it might indicate that something different than the concentrations is playing a dominating role in determining the TIP of low concertation

fungal samples. E.g., have studies found a difference in inflammatory potential of different fungal species [108, 148]. However, in the present PhD study it was not possible to determine if the species composition had an effect on the measured TIP of the samples, even though a negative correlation was found between the two dominating species *P. chrysogenum* and *A. versicolor* (r=0-0.43, p=0.01, n=24).

Further, there was a significant difference in the TIP of the aerosol samples from the dry and damp surfaces, where the dry surfaces had a significantly higher TIP ($p<0.0001$) - long sampling time with impinger being an exception (Figure 4-7). Unfortunately, the non-linear dose-response curve weakens the conclusion on this matter. However, a similar study has likewise found the TIP of samples from surfaces with low RH to be higher than the TIP of samples from surfaces with a high RH [53]. Another study found that particles released from surfaces with lower RH had a larger percentage in the respirable size fraction [51]. Together this indicates that a dried fungal infestation in a building might still be of concern for the occupants and the reason that occupants still reports symptoms of fungal growth even in environment with hidden- or dried fungal infestations [3, 14, 32].

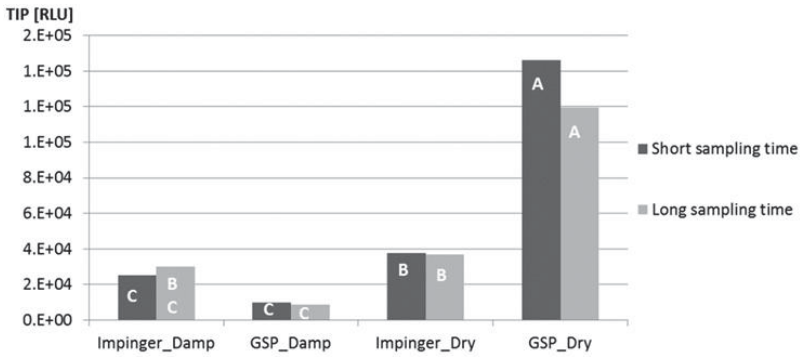

Figure 4-7 (Paper III). The total inflammatory potential (TIP) of the aerosol samples (scenario 1-4 in Table 3-2). There is no statistical difference between columns with the same letter.

CHAPTER 5. CONCLUSION

1) We hypothesised that the crawl spaces would contribute more to the pollution of the indoor air than the attics. Despite the weak foundation for the conclusion on this investigation, this did in fact prove to be true. The air transfer from the crawl spaces to the living areas was larger than from the attics. Further, the tightness of the separating structure between a construction cavity and the living area seemed to influence the air transfer between the two volumes.

2) We hypothesised that immediate action in the remediation process would reduce the exposure risk to airborne fungi. This proved to be true since drying out an infested area before substantial growth occurs, decreased the amount of fungal particles released to the indoor air. Further, *Stachybotrys chartarum* was only found on the damp surfaces, and higher species richness, as measured by imprint plate sampling, was found only on the dried surfaces.

3) We hypothesised that a higher moisture accumulation would result in an unhealthier fungal species composition of the indoor air. This proved to be true, since we found that homes with higher RH and lower ventilation rates had a higher ratio of fungi associated with mouldy buildings. This indicates that airing out is beneficial for the fungal species composition of the indoor air. Further, it was found that season influenced the species composition of the indoor air in the homes, which indicates that it is important to consider the season at which the sample is taken, when using the species composition of the indoor air as a tool for estimating the fungal burden of a building.

4) We hypothesised that even low concentrations of fungi in the indoor air, caused by fungal growth, would be inflammatory. This proved to be true, since we found an inflammatory response in the bioassay from the low fungal concentration samples from both damp and dry gypsum boards. Further, the fact that the dry surfaces still produced an inflammatory response in the bioassay indicates, that drying out a fungal infestation in a building is not sufficient to eliminate the fungal exposure.

6) We found a tendency to a J-shaped dose-response curve for the TIP versus concentration of fungi. Unfortunately this weakens the conclusions on the influence on TIP from air velocity, dampness, sampling time and sampling methods. However, this is highly relevant in connection to the interpretation of results in future inflammatory studies with environmental samples.

7) We found bacterial species on the fungal infested gypsum boards, as previous studies have done, but in the present PhD study we also found that they were not aerosolised.

CHAPTER 6. PERSPECTIVE

We are only beginning to understand the effect of specific fungal species composition on human health. Thus, more in-depth investigations on the relationship between the individual fungal species and their inflammatory potential or toxicity are highly relevant. This information will now be more achievable with the development of methods like MALDI-TOF, as a fast and accurate way to identify fungi on species level. Further, more information on fungal species level could lead to development of a regional determined ERMI or M-ERMI index, which could be a possible tool for determination of the fungal load of a building, and whether it is infested with hidden fungal growth. The present PhD study found that the M-ERMI value was affected by season, and it would therefore be relevant to investigate if the ERMI value is likewise affected by season. Thus, season might be an important consideration when using the ERMI index as a tool for determination of fungal growth in buildings.

The problematics surrounding hidden fungal growth in buildings are many; how to accurately detect presence of hidden fungal growth, how to determine the exposure risk in the living areas, and how to best remediate without substantial need for resources. Thus, investigations into temporary sealing of hidden fungal growth is an area still in need of research. The present PhD study found that air transfer from crawl spaces to living areas do happen, and the same might be the case for other construction cavities, even though the present PhD study did not find an air transfer from attics to living areas. Thus more in-depth investigations of the air transfer between construction cavities with hidden fungal growth and living areas, is needed to determine the potential exposure risk from hidden fungal growth in relevant construction cavities. The design of the ACR measurements in inaccessible cavities of the present PhD study might help finding simple ways to conduct such investigations.

Environmental samples of airborne fungi in the indoor air, often contains considerably lower concentrations of fungi than fungal samples generated in the laboratory. Thus, studies of low concentration fungal samples and their influence on human health is relevant, to better understand factors that influence the toxicity or inflammation of such samples. In the present PhD study, a J-shaped dose-response curve of fungal samples was discovered. This is an interesting discovery, which might explain why studies with low fungal concentrations often do not find association with the inflammatory potential of the samples, as studies with higher concentrations do. The present PhD study found that even low concentrations of fungal spores caused inflammation in granulocyte like cells. This is of relevance in relation to the exposure level most occupants experience in the everyday life. With the background knowledge on the J-shaped dose-response curve, it would be relevant to study the effect of low doses of fungi further, by perhaps using multiple dilutions of the samples.

The present PhD study showed that fungal infested gypsum boards released spores even after 8 hours of exposure to air jets, and that there was a difference in fungal species released after 15 min of exposure versus 8 h of exposure. This indicates that long-term studies of fungal aerosolisation from infested surfaces might be relevant to better understand the occupant's actual exposure risk. The variance in fungal spore release over time might also be of relevance in connection with choosing the optimal sampling method for fungi in infested buildings.

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Paper 1

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Assessment of exposure risk from hidden fungal growth by measurements of air change rates in construction cavities and living areas

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Abstract

The transfer of particulate and gaseous pollution from hidden fungi growing on non-visible surfaces within the building envelope to occupied rooms is limited by the separating structure. Yet, growth, even in sealed construction cavities, is known to cause annoying smells and other more adverse health symptoms among the building occupants. This study analyses limitations of air change rate measurements in inaccessible construction cavities as well as analyses of the air exchange between living areas and accessible cavities such as crawl spaces and attics. It was necessary to invent a field study technique to use the tracer gas decay method in small and inaccessible cavities. This technique allowed further investigation on the exposure risk from hidden fungal growth. Assessment of the air transfer between crawl spaces and living areas indicate that the tightness of separating structure has an influence on the exposure risk.

Keywords

Air change rate, construction cavities, hidden fungal growth, tracer gas decay, air transfer

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Introduction

Over the years, fungal growth in buildings has been known to cause various health-related symptoms among the occupants (Garrett et al., 1998; Hägerhed-Engman et al., 2009; Hulin et al., 2013; Kilpeläinen et al., 2001; Mendell et al., 2011; Tischer et al., 2011a, 2011b, 2015). When fungi are growing in non-visible and sometimes even inaccessible areas of the building, it is often referred to as hidden fungal growth. Typical locations for hidden fungal growth could be in closed construction cavities such as wall and floor cavities, but just as likely it can growth in attics and crawl spaces. The results of a Danish study by Møller and Gunnarsen (2012) indicate that up to 90% of the crawl space air travels into the living area above, making crawl spaces a significant exposure source of hidden fungal growth.

Fungal growth in buildings is caused by elevated moisture levels in the building materials (Pasanen et al., 2000). A high relative humidity (RH) combined with a thermal bridge might lead to condensation issues and thereby an increase in the material dampness. When the air temperature close to the surface drops and reaches the dew-point temperature, the moisture in the air will condensate on the surface. If the dew-point temperature is moved inside the structure, for example, by insulating an exterior wall, condensation can then happen on a non-visible surface within the building structure and lead to hidden fungal growth.

Measurements of the air change rate (ACR) are important when assessing the indoor air quality and thereby the potential exposure risk from airborne contaminants, such as fungal fragments (Engman, 2006; Hägerhed-Engman et al., 2009; Wargocki et al., 2002). Measurements of the ACR in small and closed construction cavities are necessary to assess the air transfer and thereby the potential exposure risk from these small cavities to the living area. However, conducting measurements of ACR in the field can be comprehensive, and each technique has its limitations and advantages (Bekö et al., 2016; Sundell et al., 2011). When conducting ACR measurements in buildings where the infiltration is the dominant part, for example, if a building is unoccupied and has no mechanical ventilation, the use of tracer gas techniques is suitable (Persily, 2016). There are four frequently applied tracer gas techniques: the constant-concentration method, the constant-emission method, the concentration-decay method and the PerFluorocarbon tracer (PFT) method. Studying small construction cavities presents issues different from traditional indoor air quality studies. For example, many of these cavities are inaccessible, which make it difficult to gather information on the volume and construction design, as well as measure the ACR. The volume of the cavities is a great deal smaller than the volume of a normal occupied space, which leads to limitations in practical techniques such as tracer gas measurements, where it can be difficult to control the dose and mixing. In the case of the PFT method, the small volumes can lead to incorrect results due to the vapour adsorption on the surfaces, which in small cavities present a much larger surface area in respect to the volume. To avoid layering of tracer gas, due to the differences in density between the tracer gas and the room air, a uniform mixing of the room air and tracer gas during the entire

measuring period is necessary. A way to ensure a uniform mixing will be to use constant mixing by fans throughout the measurement. However, this might result in a pressure difference that would not normally be present, thus increasing the measured ACR. When conducting tracer gas measurements, it is also important to pay attention to the type of tracer gas that is being used, since they all have different characteristics. Although CO_2 is a frequently used tracer gas, it has been documented in previous research to be a poor indicator because of the high background level and vulnerability when it comes to the presence of occupants. These factors should therefore be taken into consideration when using CO_2 as a tracer gas (Persily, 2016).

In general, the limited experience with ACR measurements in small cavities leads to a lack of knowledge on the air transfer between the cavity and the living area, thus resulting in a lack of knowledge on the exposure risk from hidden fungal growth. In this study, the concentration-decay method was chosen to investigate the ACR in attics, crawl spaces, a floor cavity and wall cavities with the purpose of evaluating the possibility of using ACR as a measure for the occupant's exposure risk to hidden fungal growth.

Methods

Description of measurement locations

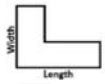
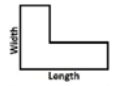
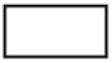
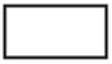
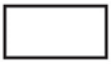
Floor separation and wall cavities are representatives of inaccessible cavities. Attics and crawl spaces represent other relevant cavities for hidden mould growth. These cavity types are often subjected to fungal growth (WHO, 2009). It was possible to gain access to a total of 12 cavities and their adjacent rooms (Table 1).

The locations were all unoccupied during the measuring period and had no mechanical ventilation, except for location W1 that had a mechanical exhaust installed, which was operating during office hours from 8 a.m. to 5 p.m. Further location W1 was the only location that was not a residence but an office. The ACR measurements were conducted outside office hours, where the mechanical exhaust was turned off. All other locations had passive natural ventilation in both kitchen and bathrooms via ducts leading up through the roof. No opening of windows

Table 1. Types and number of measuring locations and the naming that will be used throughout the article.

Cavity type	Number of locations	Naming of locations
Crawl space	7	C1–C7
Attic	2	A1 and A2
Cavity under parquet floor separation	1	F1
Cavity within gypsum partition wall	2	W1 and W2

Table 2. Overview of the geometry of the cavities and adjacent rooms.

Location	Geometry	Height (m)	Width (m)	Length (m)	Interface area (m^2)	Volume (m^3)
C1 – Room		2.30	11.60	11.70	84.40	194.00
C1 – Cavity		0.54	11.60	11.70	84.40	45.60
C2 – Room		2.30	11.60	11.70	84.40	194.00
C2 – Cavity		0.54	11.60	11.70	84.40	45.60
C3 – Room		2.40	10.00	5.06	50.60	121.44
C3 – Cavity		1.00	10.00	5.06	50.60	50.60
C4 – Room		2.40	10.00	5.06	50.60	121.44
C4 – Cavity		1.00	10.00	5.06	50.60	50.60
C5 – Room		2.40	10.00	5.06	50.60	121.44
C5 – Cavity		1.00	10.00	5.06	50.60	50.60
C6 – Room		2.30	9.00	16.30	146.70	337.41
C6 – Cavity		0.45	9.00	16.30	146.70	66.02
C7 – Room		2.35	8.82	9.81	86.58	203.72
C7 – Cavity		1.10	7.90	9.00	71.10	78.21
A1 – Room		2.35	8.82	9.81	86.58	203.72
A1 – Cavity		1.34	3.17	9.07	28.73	14.46
A2 – Room		2.30	4.00	7.25	29.00	66.70
A2 – Cavity		1.27	2.00	7.25	14.5	9.20
W1 – Room		3.33	1.10	4.09	13.60	14.77
W1 – Cavity		3.33	0.01	4.09	13.60	1.30
W2 – Room		2.20	2.80	4.50	9.90	27.72
W2 – Cavity		2.20	0.13	4.50	9.90	1.32
F1 – Room		2.50	2.80	4.00	11.20	28.00
F1 – Cavity		0.10	2.80	4.00	11.20	1.12

occurred during the measuring period. The geometry of the locations is described in Table 2.

The attics were both ventilated with openings at the end walls and ventilated along the eaves. They were also both insulated and fixed underlay; however, the attic at A2 visually had a tighter construction than the attic at A1.

The crawl spaces were ventilated in different ways. Most were ventilated with ventilation holes in the plinth, making the chimney effect a disadvantage (Figure 1). However, at two of the locations (C1 and C2), there were no holes in the plinth, as the ventilation was designed to happen via a duct leading the air from the crawl space up through the roof, making the chimney effect an advantage (Figure 2). In

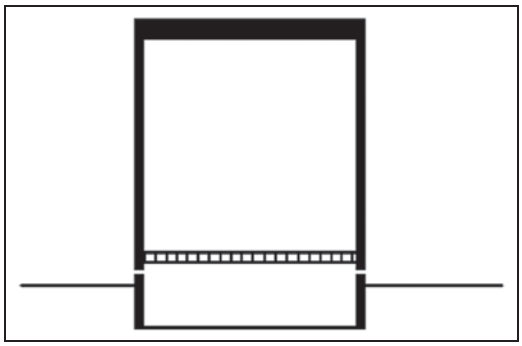


Figure 1. Conceptual sketch of the ventilation of a crawl space where the air is exchanged with the outside air through small openings in the plinth. In this situation, the chimney effect will be a disadvantage and possibly results in a lager exposure risk from the crawl space.

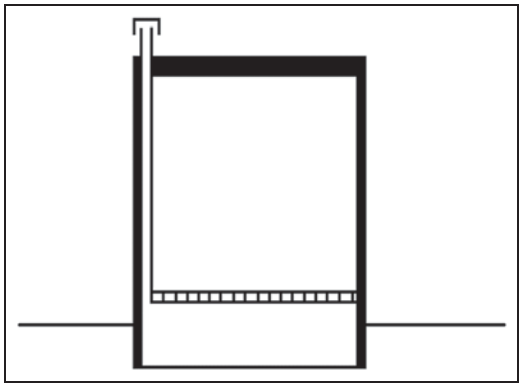


Figure 2. Conceptual sketch of the ventilation of a crawl space where the air is let out up through the roof via a duct. Here, the chimney effect is a part of the ventilation strategy and is therefore an advantage.

one of these cases (C2), the duct was dismantled, and the air was instead led directly into the bathroom above the crawl space.

The separating structure between crawl space and living area was all designed in a similar way at locations C1–C6. The structure was with no insulation between the crawl space and the living area, leaving only the parquet floor as a barrier (Figure 3). The separating structure at location C6 had a significant amount of cracks and small openings in the floor structure, separating the crawl space from the living area, making the separating structure less tight than the others. The separating structure at location C7 had a different design (Figure 3). It was insulated with mineral wool that was held in place with expanded polystyrene (EPS) boards. This resulted in a much tighter structure between the crawl space and the living area than the other locations.

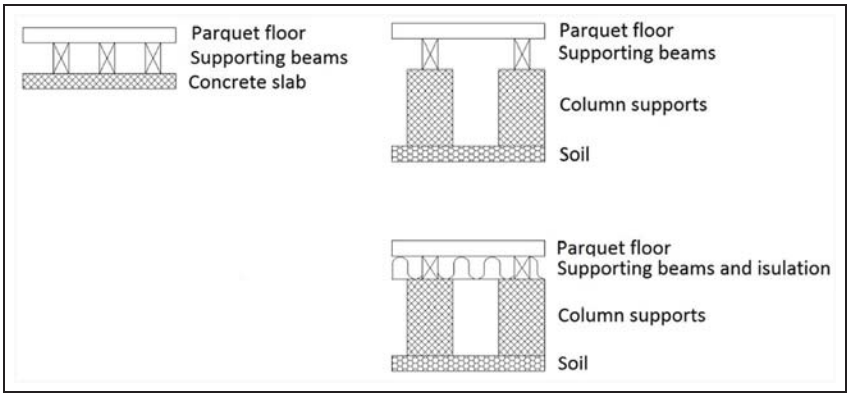


Figure 3. Left: separating structure at location F1; top-right: separating structure at locations C1–C6; and bottom-right: separating structure at location C7. Location C6 has visual cracks in the floor structure.

The floor cavity at location F1 was a joist floor on a concrete slap separating the apartments in the building. There was no mineral wool or other filling in the cavity (Figure 3). This means that the downward structure was significantly tighter than the upward structure.

Both W1 and W2 were internal gypsum walls, with a cavity between the gypsum plates with no mineral wool or other filling. The skeleton of the walls was made with perforated steel rails, which allowed the air to mix in the entire cavity.

Tracer gas measurements

In this study, a Innova 1302 Photoacoustic Tracer Gas Monitor was used to conduct the ACR measurements. The tracer gas monitors available had either a CO_2 and a SF_6 filter or a CO_2 and N_2O filter installed. Due to its accessibility, the SF_6 gas was chosen as tracer in the first part of the field study. However, it later came to our attention that the N_2O gas could be purchased in smaller and handier cartridges, and the rest of the field study was carried out using N_2O gas. By using the smaller N_2O cartridges, it was easier to control the dosing and stay below the threshold limits (SF6 = 1000 PPM, N_2O = 50 PPM; Danish Working Environment Authority, 2007; Laussmann and Helm, 2011).

The ACR was calculated using the decay regression (Sherman, 1990)

$$C(t) = C \cdot e^{-\lambda t} \tag{1}$$

where $C(t)$ is the tracer gas concentration over time (PPM), C is the tracer gas concentration (PPM), λ is the air change rate (h^{-1}), and t is the time (h).

A quantity of gas is released into the room of measurements and the decay is then measured by a tracer gas monitor. The ACR will be equal to the measured decay of the tracer gas (Persily, 2016). A sampling tube was connected to the gas

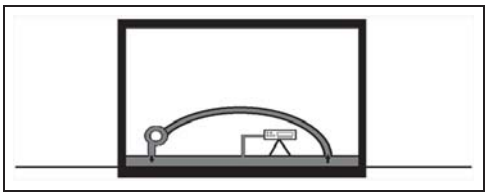


Figure 4. Conceptual sketch of the measuring set-up used for inaccessible construction cavities. A floor cavity measurement is illustrated.

monitor, and the concentration was measured approximately every 2 min. The tracer gas was dosed into the rooms or cavities using a dosing tube connected to the tracer gas. The dosing tube was fixed to a running fan, thus dosing the tracer gas into the room through the running fan, avoiding layering of the gas due to differences in density between the tracer gas and the room air.

Field study design for inaccessible cavities

In the case of small and inaccessible cavities, it was necessary to invent a way that would ensure a uniform mixing of the air and tracer gas in the cavity. This was done by constructing a sealed circulate set-up connecting a sampling and dosing tube, as well as a fan, to holes drilled into the cavity. The fan was to blow air into the cavity in one end and suck it out in the opposite end, with a force large enough to ensure air movement in all corners of the cavity (Figure 4).

The experimental set-up consisted of a large suction hose connected to the intake of a leaf blower in one end and to the cavity at the other end. The suction hose was connected to the cavity by a hole drilled in one end of the wall or floor area. Another hole was drilled at the opposite end, and here, the leaf blower outlet was connected to blow air into the cavity. A small hole was drilled close to where the air was blown into the cavity. Through this hole, the tracer gas was dosed and thereby mixed with the cavity air. Another small hole was drilled approximately in the middle of the wall or floor area, and here, the sampling tube was connected. While the leaf blower was operating, the tracer gas was dosed into the cavity for approximately 2 min. After the dosing stopped, the leaf blower was operating for another 2–3 min to ensure a uniform mixing of the cavity air. A number of pre-measurements were conducted to test that the mixing was in fact sufficient.

Since the air volume of the rooms was significantly larger than those of the inaccessible cavities, it was decided to apply constant mixing for the ACR measurements in the rooms. This means that during the ACR measurements of the adjacent room, running fans were placed around the room during the entire measuring period. After dosing the tracer gas into the room, the air and tracer gas were allowed to mix for 5–10 min before the measurement was started.

At locations C1 and C2, measurements with and without constant mixing were conducted in order to analyse the potential increase in the ACR caused by the pressure difference that will occur when using constant mixing. The actual pressure difference was not measured.

Field study design for accessible cavities

For the crawl spaces and attics, constant mixing by fans was chosen equal to the study design for the adjacent rooms. Fans were placed strategically around the crawl space or attic, and the entrance was sealed off. A dosing tube, through which the tracer gas was released, was fixed to one of the fans in the cavity. The sampling tube was fixed to a construction part approximately in the middle of the cavity. For the crawl spaces and attics, the fans were mixing the air for approximately 5–10 min before the measurement was started, equal to the measurements in the adjacent rooms.

For locations C1 and C2, the ventilation of the crawl spaces was sealed off, and the ACR measurements were repeated to investigate the difference between ventilated and non-ventilated crawl spaces. For location A1, the ventilation of the attic was reduced by sealing of the ventilation openings at the end walls and the ACR measurement was repeated.

Measurements of air transfer

In a few selected locations (A1, A2, C6 and C7), measurements of air transfer from cavity to living area were conducted to get an indication of the emission rate. This was done manually by continuously dosing tracer gas into the cavity, while the tracer gas monitor was measuring the concentration in the cavity and living area, respectively. This way, it was ensured that the cavity had a significant higher level of tracer gas during the entire measuring period compared to the background level, thus making it possible to measure the amount of tracer gas transferred to the adjacent room. However, it should be borne in mind that even though the concentration in the crawl space was kept higher than the background level, it was not constant throughout the measuring period, which will present inaccuracies when calculating the emission rate.

Results and discussion

In general, the measured ACR levels in the living areas were low and in fact did not live up to the required ventilation demands of $0.5\,h^{-1}$ stated in the National Danish Building Code (Tables 3–5). This is in agreement with a previous study of 390 Swedish homes, where the measured ventilation rate likewise did not live up to the demands in the National Swedish Building Code (Bornehag et al., 2005).

A reason for the low ventilation rates measured in this study might be that the buildings were all unoccupied during the measuring period, and therefore, no

Table 3. Results of the crawl space investigations at C1–C7.

	Air change (h^{-1})	Flow (l/s)
C1		
Adjacent room with constant mixing	0.20	10.78
Adjacent room without constant mixing	0.13	7.01
Crawl space with constant mixing	0.28	3.55
Crawl space without constant mixing	0.78	9.88
Crawl space with constant mixing and without ventilation	0.71	8.99
Crawl space without constant mixing and without ventilation	0.33	4.18
C2		
Adjacent room with constant mixing	0.34	18.32
Adjacent room without constant mixing	0.25	13.47
Crawl space with constant mixing	0.72	9.12
Crawl space without constant mixing	0.56	7.09
Crawl space with constant mixing and without ventilation	0.55	6.97
Crawl space without constant mixing and without ventilation	0.52	6.59
C3		
Adjacent room with constant mixing	0.77	25.97
Crawl space with constant mixing	1.19	16.73
C4		
Adjacent room with constant mixing	0.86	29.01
Crawl space with constant mixing	2.27	31.91
C5		
Adjacent room with constant mixing	0.49	16.53
Crawl space with constant mixing	0.77	10.82
C6		
Adjacent room with constant mixing	1.44	134.96
Crawl space with constant mixing	1.45	26.59
C7		
Adjacent room with constant mixing	0.28	15.84
Crawl space with constant mixing	0.77	16.73

The measured ACR and the calculated flow are listed.

windows or doors were opened. Furthermore, the results of the ACR measurements in the living areas show that the ACR increased when constant mixing was applied (Table 3). This means that the measured ACR results might be overestimated in this study.

Crawl spaces

Table 3 presents the results of the ACR measurements conducted in the crawl spaces at locations C1–C7. The results of the ACR measurements in the crawl space did not give any clear indication as to whether the ACR in the crawl spaces would increase further under constant mixing if the ventilation holes were free and open. The reason for the lack of consistency in these results could be due to

Table 4. Results of the attic investigations at A1 and A2.

	Air change (h^{-1})	Flow (l/s)
A1		
Adjacent room with constant mixing	0.28	15.84
Attic with constant mixing	7.6	30.53
Attic with constant mixing and reduced ventilation	3.29	13.21
A2		
Adjacent room with constant mixing	0.36	6.67
Attic with constant mixing	43.2	110.4

The measured ACR and the calculated flow are listed.

Table 5. Results of the wall and floor investigations.

	Air change (h^{-1})	Flow (l/s)
W1		
Adjacent room with constant mixing	0.23	0.94
Partition wall without constant mixing and with ventilation in adjacent room	0.97	0.35
Partition wall without constant mixing and without ventilation in adjacent room	0.64	0.23
W2		
Adjacent room with constant mixing	0.12	0.92
Partition wall without constant mixing	0.19	0.17
F1		
Adjacent room with constant mixing	0.1	0.78
Floor cavity without constant mixing	0.05	0.02

The measured ACR and the calculated flow are listed.

spontaneous weather change, such as wind speed. The measurements of the crawl space at C1 with and without using constant mixing stand out the most. The logbook shows that the measurements were conducted over a 2-day period, where the weather conditions changed significantly with regard to both wind speed and temperature. Studies have shown that the weather conditions have a significant influence on the ACR in buildings (Bekö et al., 2016; Laussmann and Helm, 2011; WHO, 2009).

Attics

Table 4 presents the results of the ACR measurements conducted at locations A1 and A2. The results show that the ACR in the attics is higher than in the other types of construction cavities, as well as the adjacent rooms. This was expected because attics are often constructed as well-ventilated cavities. The results show that the

ACR decreased significantly when the ventilation of A1 was rescued, although still higher than in the adjacent room and other cavities measured in this study.

Wall and floor cavities

Table 5 presents the results of the ACR measurements conducted in the inaccessible cavities of the walls and floor separation. The results of the ACR measurements conducted at W1 show that the ACR of the adjacent room had an influence on the ACR in the wall cavity. If the mechanical exhaust in the adjacent room was operating, the ACR in the cavity increases. This indicates that some of the air from the cavity is ventilated through the adjacent room creating an exposure risk for occupants.

The ACR measured in the floor cavity F1 was approximately half of the ACR measured in the adjacent room. This could be expected, since there was no occupancy activity in the room, which would likely have conducted a pump-effect that would increase the air movement in the floor cavity.

Air transfer from crawl space and attics to the living area

The measurements of tracer gas transferred from the crawl space to the living area are shown in Figure 5. The results show that for C6, the air transfer is significantly larger than for C7 and an actual build-up of tracer gas is measured. There is no indication of a build-up of tracer gas in the adjacent room for location C7; however, the measured tracer gas level in the living area is higher than the background level.

The measurements of tracer gas transferred from the attic to the living area are presented in Figure 6. For both attics there were no measurable transfer between the attic and the living area. The measured values were all on level with the measured background level.

The graphs in Figures 5 and 6 show a maximum air transfer from attics A1 and A2, respectively, at <1 PPM/h and <0.5 PPM/h, as well as a maximum air transfer from the crawl space at C7 to the living area at <2.7 PPM/h. For the crawl space at C6, the graphs show an approximate air transfer for the crawl space to the living area around 40 PPM/h.

In general, the measured values in the adjacent rooms of the attics at A1 and A2 are at a level that might only be due to the uncertainties of the equipment. For the crawl space at C7, the measured tracer gas level in the living area is low, but higher than for the attics and above background level, which indicates that some air transfer happens between the crawl space and living area.

For the crawl space at location C6, a build-up of tracer gas in the adjacent room was measured. Thus, a calculation of the emission rate can be conducted.

The graph on the left side of Figure 5 indicates that steady state is not yet achieved when the measurement was stopped. However, the graph also indicates that the curve is flattening and therefore nearing steady state. To calculate the accurate emission rate from the crawl space to the living area, a constant

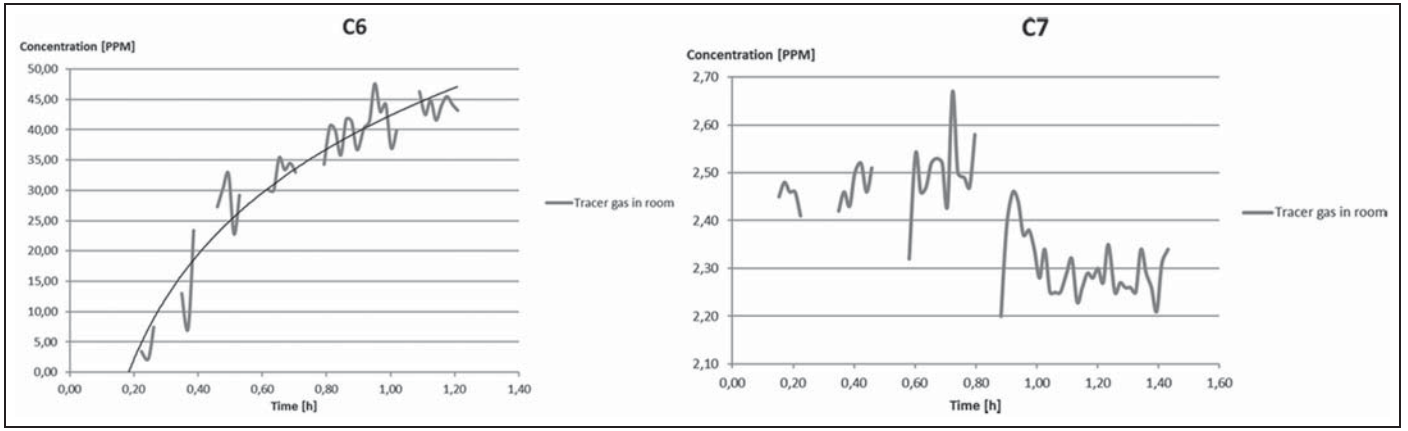


Figure 5. Left: the results of the measurements of tracer gas transfer from crawl space to living area at C6. Right: the results of the measurements of tracer gas transfer from the crawl space to the living area at C7.

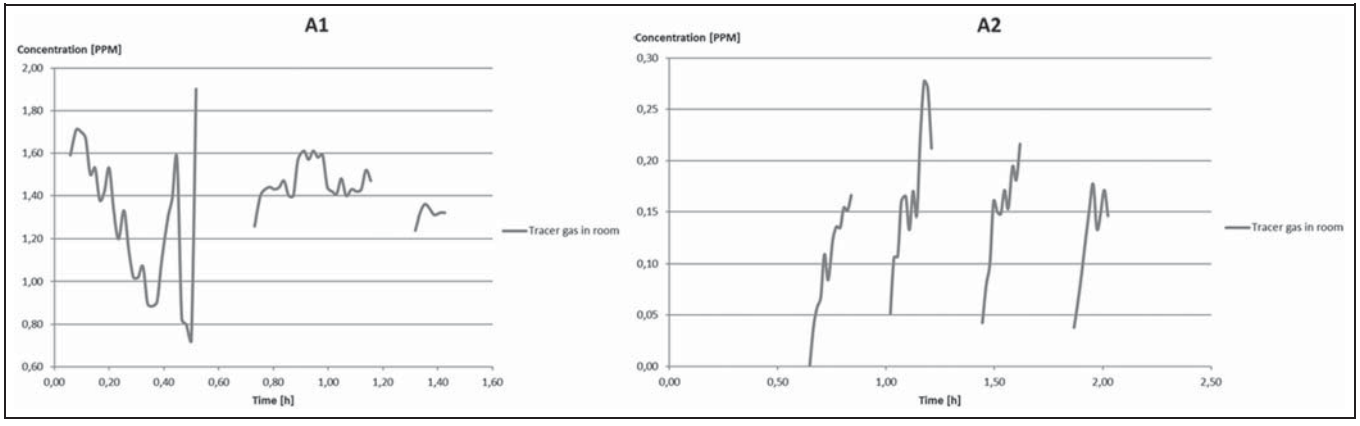


Figure 6. Results of the tracer gas transferred from the attic to the living area: location A1 (left) and location A2 (right).

concentration of tracer gas in the cavity should be applied and steady state should be reached.

The flowing equation describes the curve of the build-up process of tracer gas in the room air

$$C = \frac{G}{(n \cdot V)} \cdot (1 - e^{-n \cdot t}) \tag{2}$$

where C is the concentration in the room at time t (PPM), N is the ACR (h^{-1}), T is the time variable (h), V is the room volume (m^3), and G is the emission rate (PPM/h).

A calculation of G for every measuring point was made, and the average value is converted from PPM/h to m^3/h using the following equation

$$\frac{G}{10^6} \cdot V = \dot{\nu} \tag{3}$$

where $\dot{\nu}$ is the air flow of gas from the crawl space to the adjacent room (m^3/h).

This calculation gives an air flow from the crawl space at C6 to the adjacent room at 8.24 m^3/h. Because only the decay method was chosen to conduct the ACR measurements in this study, limitation in the investigation of the emission from the cavities to the living areas must be acknowledged. The constant-concentration method would have been appropriate for such an investigation, and the calculation of the emission rate from the crawl space to the living area at C7 is therefore rather simplified and should only be regarded as an estimation. A limitation of the study is therefore that only one tracer gas method was used to determine the ACR and the emission rates. Studies have shown a significant deviation between the results of the ACR when using different measuring techniques (Bekö et al., 2016; Kolarik et al., 2016). It is therefore not without loss to conduct simplified ACR measurements in the field.

There is a clear indication that the design and tightness of the construction, separating the cavity from the living area, play an important role in the exposure risk from construction cavities. In fact, the results show that the exposure risk from the well-insulated crawl space at C7 is only slightly larger than the exposure risk from the attics A1 and A2. Thus, when evaluating the exposure risk of hidden fungal growth, it is necessary to take into consideration not only the location of the hidden fungal growth but also how tight the construction between the infected cavity and the adjacent room is.

Further investigations on the influence of the separating construction are necessary to get a better understanding of the risk of exposure from hidden fungal growth. A laboratory study by Airaksinen et al. (2004) found that the amount of cracks in the floor did not have a significant difference on the measured viable fungal spores in the adjacent room. The contradiction might be because this study is a field study as opposed to a laboratory study, and that in this study, the air transfer is assessed by measuring the tracer gas transfer and not the amount of viable fungal spores. However, this indicates that further study on the exposure risk from particulate and gaseous pollution and its driving factors are necessary.

Conclusion

Further investigations on the air exchange between living area and construction cavities are required. However, some conclusions from this study can be made as follows:

- This study shed light on the limitations and challenges regarding ACR measurements of small and inaccessible construction cavities.
- The developed experimental set-up for measuring ACR in closed construction cavities proved suitable.
- The results also demonstrate that the design of the separating structure is of significant importance to the air exchange between living area and construction cavity.

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Paper 2

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Airborne fungal species associated with mouldy and non-mouldy buildings – effects of air change rates, humidity, and air velocity

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ABSTRACT

Several studies have shown an association between dampness and health issues like headache and asthma. To better understand the exposure risk of fungal growth in buildings this study investigates the release of fungi from gypsum boards infested with fungi from a moisture-damaged house. Further, the composition and concentration of fungal species in indoor air of five non-moisture-damaged homes are analysed, and the ratio between species associated- and not associated with moisture-damaged buildings are related to air change rate (ACR) and relative humidity (RH). The air velocity near the surface of the gypsum boards in combination with the changes in sampling time influenced the particle release rate. After 8 h particles were still released, and more species were released during 8 h with low air velocity than during 15 min with high air velocity. More fungal species and a higher release rate were found from damp surfaces with substantial growth than from gypsum boards dried out before they were totally colonized. In the five homes ACR and RH had a significant influence on the fungal species composition. Thus, a low ACR and a high RH were associated with increased ratio of species associated with moisture-damage relative to species not associated with moisture-damage. In conclusion, increasing the ventilation and reducing the RH of the indoor air will have a beneficial effect on the airborne species composition. Further, fast action by drying out a fungal infestation has a positive impact on the exposure risk in terms of exposure level and species composition.

1. Introduction

Several studies have shown association between dampness or visible fungal growth in buildings and health issues such as colds, fatigue, headache, concentration difficulties, and on a more severe level, asthmas and allergies [1–4]. To better understand the exposure risk of fungal growth in buildings, a fungal index, ERMI (Environmental Relative Mouldiness Index), has been developed based on fungi in homes in the US. The ERMI value is a ratio between common species associated with (Group1) and common species not associated with (Group 2) moisture-damaged buildings in the US [5]. Higher ERMI values have been found in homes of asthmatic children [6,7] and adults [8]. Likewise, a French study found a significant positive correlation between higher ERMI values and measures of fungal growth in buildings [9]. Air or surface dust samples are often used to identify the potential presence of fungal growth in a building, especially if it cannot be revealed by a visual inspection, e.g. if the fungal growth is located on a non-visible surfaces within the building structure [10]. Also the ERMI index is based on dust samples, and ERMI values are shown to correlate with measures of airborne fungi [11]. Recently, it has been shown that fungal species in the ERMI index are also common in airborne settled dust sampled by the electrostatic dust collector (EDC) in Danish homes [12], and concentrations of fungi sampled by the EDC correlates with fungal concentrations in airborne inhalable dust [13]. In some homes, indoor concentrations of fungi follow the outdoor concentration [14–16], which are affected by season and ventilation rate [14,16]. Several studies have found poor association between observed dampness and total fungal DNA on door frames [17] as well as between concentration of airborne fungi and visible fungal growth [10,14,18,19]. A reason for this might be the entrance of outdoor fungi contributing to what is released from the indoor infested surfaces. Identification of fungal species may help the understanding of factors affecting the occupants' exposure risk to airborne fungi.

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Fungi need damp conditions and organic material to initiate growth and proliferate in buildings. Elevated moisture levels in building materials can occur for several reasons. One could be a water-damage from e.g. a leaking installation; another could be condensation of the room air on cold surfaces within the building. When elevated moisture levels in building materials are caused by condensation, the moisture level varies depending on the temperature and relative humidity (RH) [20], thus resulting in periods with varying conditions of dampness or dryness in the same area. This may have an influence on the fungal growth process [21] in the area, and thereby on the occupants' exposure risk. Many building materials contain organic substances, e.g. the cardboard sandwiching the gypsum in a gypsum board. Gypsum boards are commonly used in buildings for wall or ceiling constructions. Gypsum boards are sensitive to damp conditions in the indoor environment since they easily absorb water. Further, they contain cellulose which support fungal growth [22]. These might be the reasons that gypsum boards often have been used in investigations of fungal growth on building materials and spore release [23–27].

Most studies of fungal growth on building materials have used a monoculture known to grow in moisture-damaged buildings [23,25,27]. However, in buildings, both with and without moisture-damage, several fungal species are present in indoor air [5,28], thus both occupants and damp surfaces are exposed to several fungal species. This may be of importance for the fungal growth on building materials and the following release from infected surfaces. The release rate of fungal particles from fungal colonised gypsum boards depends on the species [27], the dampness of the surface [24], and the air velocity near the surface [12,25,27]. In buildings, the air velocity near surfaces varies. For example, visible surfaces in occupied rooms are often exposed to a larger air velocity than surfaces in non-ventilated and sealed construction cavities. Likewise, the air velocity near surfaces behind furniture or in corners will usually be lower than near free and open surfaces in the room. Most studies on the effect of air velocity on the fungal release from fungal infested surfaces are conducted over a short time spand of 1–5 min [12,24–26]. However, most occupants spend up to 60–90% of their time indoors [29], resulting in a considerable longer exposure time. To better understand the occupant's exposure risk to airborne fungi it is therefore important to gain knowledge on the effect on long-term release of airborne fungi in buildings.

The aim of this study was to gain knowledge on the exposure risk of airborne fungal species associated- and not associated with water-damage. We have investigated the exposure risk from a fungal infested surface, by laboratory simulation of a water-damage leading to fungal growth on gypsum boards. To make it as representative as possible, the gypsum boards were inoculated with a mixture of fungal species sampled in a moisture-damaged house. The effect of reducing the moisture level in the infested gypsum boards was studied as well as the effect of varying sampling time and the air velocity near the surfaces. Further, the species composition of airborne fungi in five non-moisture-damaged homes were analysed against the ERMI index, and the effect of air change rate (ACR) and RH was studied.

2. Method

2.1. Design of the fungal release study

Investigation of the aerosols from the infested gypsum boards included four scenarios of both measurements of particles and sampling of fungal spores (Table 1). Three repetitions for each scenario were prepared. Further, surface scrapings and contact plates were each conducted on a total of 6 damp and 6 dry surfaces.

Table 1
Release scenarios with fungal release from damp and dry gypsum boards. Three repetitions for each scenario were prepared, and both sampling time and air velocity was varied to insure that the infested gypsum boards were affected by the same air volume across the four scenarios.

Scenarios	Description of scenarios
1	APS measurements and GSP sampling at 2.5 m/s for 15 min over damp surfaces
2	APS measurements and GSP sampling at 9.0 m/s for 8 h over damp surfaces
3	APS measurements and GSP sampling at 2.5 m/s for 15 min over dry surfaces
4	APS measurements and GSP sampling at 9.0 m/s for 8 h over dry surfaces

APS = Aerodynamic Particle Sizer, GSP = GesamtStaubProbenahme.

Air velocity and sampling time is varied simultaneously to ensure that the infested gypsum boards were affected by an equal air volume across the scenarios (Table 1, scenario 1–4).

The scenarios were conducted in series, leaving a rather significant time gap in between them, where growth was allowed to continue on the boards referred to as boards with damp surfaces (Figure 1). The damp and dry surfaces did therefore not have equivalent growth. The GSP (GesamtStaubProbenahme) sampling was conducted firstly, the APS (Aerodynamic Particle Sizer) measurements secondly, the surface scrapings thirdly, and lastly the contact plates (Figure 1). The sampling by GSP and measurements with the APS were conducted to on different surfaces. Likewise, the scrapings and contact plate samplings were conducted on different surfaces. Particle concentrations were used as measure of the release of fungal aerosols, as it was assumed that fungal particle was the primary aerosols released from the infested gypsum boards.

2.2. Sampling of fungi from moisture-damaged house

The EDC was used for sampling of wild flora fungi in a moisture-damaged house. The sampling was conducted over a two-week period in October 2014 and two EDCs were placed in different rooms of the house. The EDC consisted of four electrostatic cloths (Zeeman Alphen, Netherlands) with an exposure area of 0.0209 m^2 each (11 × 19 cm). In the laboratory, the dust on the EDC cloths was extracted in 20 ml extraction liquid (0.05% Tween20 solution) (SIGMA-ALDRICH, USA) by shaking at 300 rpm for 1 h.

2.3. Sampling of fungi in non-moisture-damaged homes

The species composition of dust sampled by EDCs from five non-water-damaged homes for each season: summer, autumn, winter, and spring, was analysed. The homes were labelled A-E, and out of the five homes there were three detached houses (A, B and D), one town house (C), and one apartment (E). All samples were taken in the living room of the homes. Home E was the only home with mechanical ventilation. The ACR of all five homes were measured using the constant concentration method. The results and a detailed description of the ACR measurements can be found in Frankel et al. [16]. Likewise, the RH was measured in all five homes using a TinyTag Plus Data Loggers (Gemini Data Loggers, UK) [16].

2.4. Inoculation on gypsum boards

Sterilised gypsum boards were moisturised in a sealed and sterilised environment before inoculation with fungi from the EDC sampling of the moisture-damaged house (Figure 2).

To simulate the situation of a water-damage in a building,

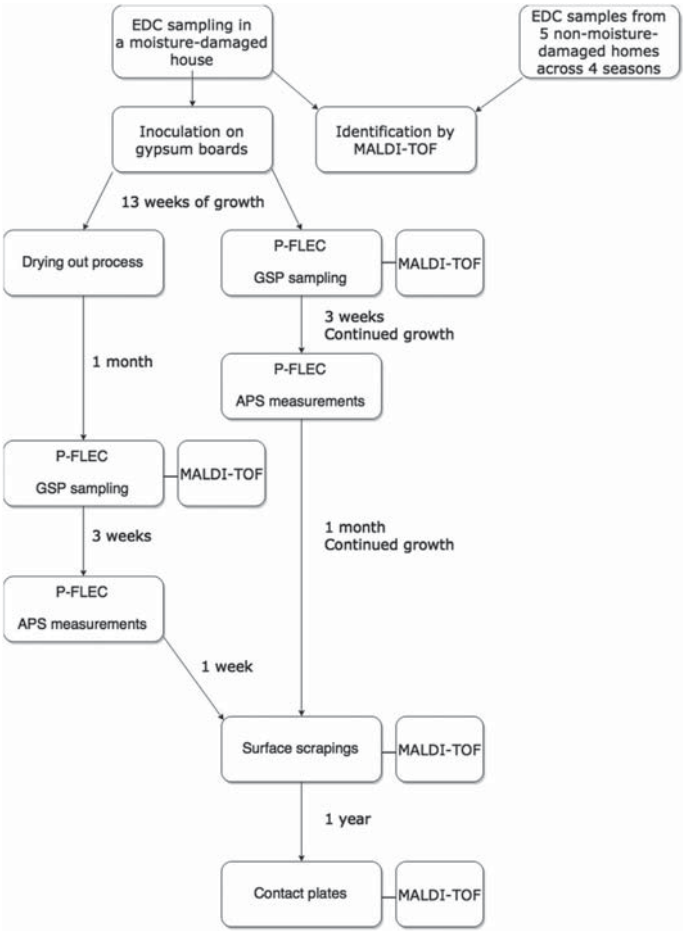

Fig. 1. Flow diagram of the sampling and measurements conducted in the present study. The diagram shows an approximate timeline for the sampling and measuring process in the laboratory. EDC = Electrostatic Dust Collector, MALDI-TOF = Matrix-Assisted Laser Desorption/Ionization-Time-Of-Flight, P-FLEC=Particle-Field and Laboratory Emission Cell, GSP = GesamtStaubProbenahme, APS = Aerodynamic Particle Sizer.

gypsum boards of 14 × 14 cm were set to soak until saturation in a sterilised aluminium box. Three boards were placed in each box with 400 ml distilled Milli-Q water for approximately 2 h. The gypsum boards were inoculated with 20 ml of the extraction suspension from the ECD, and the boxes were sealed by use of a rubber band between the box edge and a glass lid. To keep the RH high during the growth process, two petri dishes were placed at the bottom of the box, each containing 75 ml saturated K_2SO_4 saturated solution [24] (Figure 2). A data logger (EasyLog EL-USB-2, Lascar electronics, UK) was placed in each box logging the temperature and RH during the entire experiment. Data from the loggers show that the RH in the boxes was just below 100% throughout the experiment. They were incubated at room temperature (approximately 20 °C) and covered with white linen [27]. After 13 weeks, growth was visible on the surface of all gypsum boards and the RH was lowered in one-half of the boxes by adding 666 g of Silica Gel per gypsum board [12,24]. The Silica Gel absorbed the moisture in the air and thereby made the RH decrease to around 35–40%, thus stopping the growth. All boards had visual fungal growth, but not all boards were overgrown, and there was a visual difference in the growth between boards.

The three infested gypsum boards in each box were used for different samplings; GSP, APS, and Impinger (BioSampler, SKC, UK) (the results of the Impinger sampling is used in another study). The repetitions where conducted on gypsum boards incubated in different boxes to insure resemblance between the particle measurements and the samplings of fungi.

2.5. P-FLEC measurements

The P-FLEC (Particle-Field and Laboratory Emission Cell, Chematec, Denmark) was used for measuring the release of airborne particles from the gypsum boards. A bar with ten 0.8 mm nozzles was rotated 1.0 cm over the surface; one complete rotation was 60 s. The air jet was directed towards the surface at an angle of 45°, and scanned a circular area of 130 cm^2. A detailed description and illustration of the device can be found in Kildesø et al. [27]. The aerosols were transported by the airflow in the chamber to the

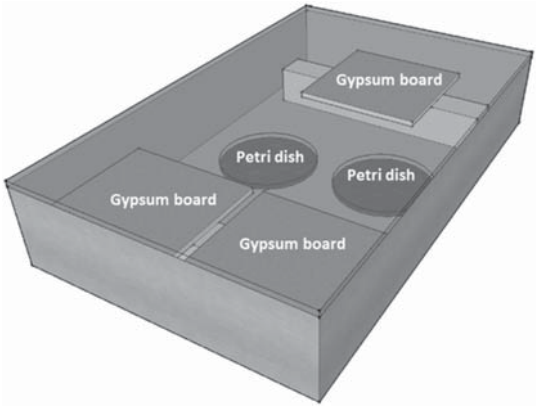

Fig. 2. Illustration of the experimental set-up of inoculation and growth on gypsum boards. Two glass Petri dishes with 75 ml saturated K_2SO_4 solution was used to keep a high relative humidity (RH) in the boxes. Three gypsum boards were placed in each box.

outlet at the top [25–27]. Here, either an APS device or a GSP sampler was connected to, respectively, measure the particles or sample the fungal spores released from the surfaces. Measurements were conducted over a period of 8 h with an air velocity of 2.5 m/s, referred to as long-term measurements with low velocity, and 15 min with an air velocity near the surfaces of 9.0 m/s referred to as short-term measurements with high velocity. By varying both air velocity and sampling time an equal air volume was insured across the scenarios. For the short-term measurements, the P-FLEC was moved slightly over the surface every 2 min and for the long-term measurements it was moved slightly every 30 min. This was done to cover as large as possible an area of each gypsum board.

2.6. APS measurement

The particle size and amount released from the surfaces of the gypsum boards was measured by the APS device (APS 3320, TSI Inc., USA) as described by Kildesø et al. [27]. The particles were measured every second in 51 size ranges between 0.54 μm and 19.8 μm.

2.7. GSP sampling and particle extraction

The fungal particles released from the surfaces of the gypsum boards were collected onto a polycarbonate filter with pore size 1 μm (Frisenette, USA) using a GSP sampler (CIS by BGI, INC, USA). The GSP samples at a flow of 3.5 l/min. The polycarbonate filters were extracted in 5.6 ml 0.0005% Tween20 solution. The filters were set to shake at 300 rpm for 1 h. The suspension was plate diluted on DG18 ager plates (MERCK, Germany) for cultivation and identification. The DG18 agar was chosen since it has been proven to be more effective than other agars both in regards to quantity and species richness [30].

2.8. Surface scraping

The scraping of the surfaces was made by gently scraping a sterilised and sharp blade over the surface of the infested gypsum boards. The entire area of the gypsum board (196 cm^2) was scraped. The scrapings were suspended in 5.6 ml 0.0005% Tween20 solution, before dilution on DG18 agar for identification. Scraping was done to get an estimation of the fungal species growing on the surface compared to what was released and captured on the GSP filter.

2.9. Sampling with contact plates

Surface sampling with contact plates was conducted one year after the other samplings and measurements (Figure 1) and on surfaces that had not been used for scrapings. This sampling was done to investigate the difference in species recovery after one year if the surfaces were left to dry out naturally or forced to dry early in the growth process. At that point, the damp surfaces had dried out and will therefore be referred to as previously damp surfaces in connection to the sampling by contact plates. The contact plates were made with DG18 agar like the other agar plates used in the study. They were similar to the contact plates often used by practitioners investigating fungal infested buildings. The contact plates were made by The Danish Technological Institute. The contact plates were pressed against the surfaces of the infested gypsum boards and placed in an incubator at 20 °C. After 3 days, growth was visible and colonies separable, thus suitable for identification.

2.10. Identification of fungi

For identification of fungi found in the samples, the MALDI-TOF analysis (Matrix-Assisted Laser Desorption/Ionization-Time-Of-Flight) was performed on an Autoflex III (Bruker Daltonics, Germany). The ethanol extraction method was used for identification, following the manufacturer's protocol. A small part of the fungi was added to Sabouraud Liquid Medium (Oxoid, UK) and incubated at 25 °C under constant shake at 300 rpm for at least 12 h or until visible biological material are produced. The biological material was separated from the growth medium and washed twice in distilled Milli-Q water. After washing of the pellet, it was suspended in 1000 μl of 75% ethanol and vortexed for at least 30 s. The pellet was separated from the ethanol and set to dry for 30–60 min. The size of the pellet was estimated to determine the amount of formic acid (SIGMA-ALDRICH, USA) and acetonitrile (SIGMA-ALDRICH, USA) to be added when the pellet had dried. After adding formic acid and acetonitrile, the sample was centrifuged at 13,000 × *g* for 2 min and 1 μl of the supernatant was spotted onto the ground steel target plate (Bruker Daltonics, Bremen, Germany). The droplet was set to dry at room temperature and then overlaid by 1 μl of matrix (Bruker Daltonics, Germany). When the matrix had air dried, the sample was ready for analysis. A score below 2.0, means that the identification is successful on both genus and species level. The standard commercial available fungal MALDI-TOF MS database included 25 of the 36 species from the ERMI index and additional two species was available in the data based used in the present study.

2.11. Data treatment

To compare the particle data measured with the APS, the geometrical mean diameter (D_g) was calculated using the following equation [12]:

$$D_g = \left(D_1^{n_1} \cdot D_2^{n_2} \cdot D_3^{n_3} \ldots D_n^{n_n}\right)^{1/N} \quad (1)$$

Where:

D_g is the geometrical mean diameter
D_1 is the geometrical midpoint of the interval
n_1 is the measured number of particles in the interval
N is the total number of particles summed over each interval.

The geometrical mean uses the product of the values and thereby present the typical value or central tendency of the particle sizes in the data set, making them comparable in a more manageable way.

The ERMI value can be calculated by the following equation [5,7]:

$$ERMI\ value = \sum_{i=1}^{26} \log_{10}(s_{1i}) - \sum_{j=1}^{10} \log_{10}(s_{2j}) \quad (2)$$

Where:

S_1 is the sum of $\log_{10}$ of the concentrations of group 1 fungi measured in the homes.
S_2 is the sum of $\log_{10}$ of the concentrations of group 2 fungi measured in the homes.

In the present study, the calculated ERMI values will be referred to as M-ERMI values since the species identification has been made by use of MALDI-TOF MS analysis. The database used for identification contained 27 of the 36 species included in the ERMI index.

The statistical analyses are all conducted in the Statistical Analysis Software 9.4 (SAS). The values were log transformed, and for comparison of the effect of air velocity in combination with sampling time on the particle release rate and total particle release, the analysis of variance procedure was used (Proc Anova). For the correlation analyses of the effect of ACR and RH on the total concentration of airborne fungi as well as M-ERMI values for the five homes, the Proc Corr command was used. The M-ERMI values as affected by season were compared by Proc Mixed model with random effect of home.

3. Results

3.1. Fungal species

A total of 17 fungal species were found in the EDC samples of the moisture-damaged house, and four of these species were recovered

Table 2
Fungal species found in the five homes, inoculum for the gypsum boards, the release scenarios, and surface samples.

	Homes n = 5	Inoculum	Damp				Dry			
			Air		Surface		Air		Surface	
			High velocity	Low velocity	Scrape	Contact plates	High velocity	Low velocity	Scrape	Contact plates
Aspergillus										
A. flavus[a]	+	+						+		
A. fumigatus[a]	+	+		+						
A. glaucus	+									
A. niger[a]	+					+				
A. nidulans	+					+				
A. penicilloides[a]	+									
A. ustus[b]		+			+	+				
A. versicolor[a]	+	+	+	+	+		+		+	
Botrytis										
B. cinerea		+								
Candida										
Ca. lambica[a]	+									
Ca. sorbosa[a]	+									
Chaetomium[c]										
Ch. globosum[a]	+	+							+	
Cladosporium										
C. cladosporides[b]	+	+								
C. herbarum[b]	+	+								
Epicoccum										
E. nigrum[b]	+									
Fusarium										
F. proliferatum	+									
Penicillium										
P. dierckxii		+								
P. brevicompactum[a]	+	+								
P. camemberti	+									
P. chrysogenum[b]	+	+	+	+	+	+	+	+	+	+
P. citrinum	+									
P. commune[b]		+								
P. corylophilum[a]	+	+								
P. glabrum[a]	+	+							+	
P. olsonii	+	+								
P. roqueforti	+									
P. verrucosum		+								+
Stachybotrys										
S. chartarum[a]		+								
Wallemia										
W. sebi[a]	+									

The table is divided into damp and dry surfaces and in the cases of high velocity it should be noted that the sampling time was 15 min whereas in the cases of low velocity it should be noted that the sampling time was 8 h.

[a] Fungi frequently found in US homes and associated with mouldy buildings (ERMI Group 1).
[b] Fungi frequently found in US homes but not associated with mouldy buildings (ERMI Group 2).
[c] Yeast.

in aerosols sampled with the GSP and seven in surface samples from the gypsum boards (Table 2). On the contact plates taken on the previously damp surfaces, four out of the 17 species were recovered. On the contact plates taken on the surfaces dried out by use of Silica Gel, two out of the 17 species were recovered. A visual inspection of the gypsum boards revealed the growth of a *Coniophora puteana*-like fungus on many of the surfaces randomly selected for GSP sampling and used for identification. The results show a remarkable smaller amount of aerosols from the *Coniophora puteana*-like infested surfaces. *Penicillium chrysogenum* was the most dominating species and the only species found in all release scenarios and surface samples (Table 2). *Aspergillus versicolor* was found in three out of the four scenarios, in two out of the four surface samples, in the home dust samples, and in inoculum from the moisture-damaged house. *Stachybotrys chartarum* was discovered by a visual inspection on the damp surfaces, but on none of the dry surfaces. *Chaetomium globosum* was only found in the scrapings of the dry surfaces and in none of the aerosols. A visual inspection of the gypsum boards confirmed that *Ch. globosum* was in fact only visible on the dry surfaces. Short-term sampling released only *P. chrysogenum* and *A. versicolor*, whereas the long-term sampling furthermore released *Aspergillus fumigatus* and *Aspergillus flavus*.

3.2. The effects of ACR, RH and season on the concentration and composition of fungal species

A calculation of the geometrical mean value (GM) of the ACR across all four seasons in each home shows that home A, C, and D did not apply with the required ventilation rate of 0.5 h^{-1} stated in the National Danish Building Code [31]. A positive correlation ($r = 0.667$, $P = 0.0018$ $n = 19$) was found between the ACR and the total concentration of airborne fungi sampled in the five non-moisture-damaged homes.

The M-ERMI values ranged from −6.60 to 13.01 (Table 3), and a significant negative correlation between ACR and the M-ERMI values ($r = -0.689$, $P = 0.0011$ $n = 19$) was found. Further, the M-ERMI values were higher during autumn and winter than during summer. A statistical mixed-model analysis showed a significant effect of season on M-ERMI values ($P = 0.0002$) (Table 3 two bottom rows) with lower values in winter and autumn compared to summer and spring. The GM of the RH across all four seasons showed that the RH in the homes is between 48% and 56%. A statistical analysis showed a significant positive correlation between RH and M-ERMI values ($r = 0.527$, $P = 0.024$, $n = 18$). The total concentration of airborne fungi was not affected by the RH ($P = 0.96$). A stepwise regression with all three factors ACR, RH, and season, show a significant effect of ACR (Estimate = −6.08, $p = 0.0005$) and RH (Estimate = 32.96, $P = 0.007$).

The total concentrations of fungi for each home during each season are presented in Table 4. A significant effect of season was found ($P < 0.0001$) with the highest concentrations during the summer. During the winter season, the concentration of airborne fungi in home E was below the detection level.

3.3. Fungal particle release

The air velocity near the surface in combination with the changes in sampling time had a statistical significant influence on the fungal particle release rate ($P = 0.0003$, $n = 10$) and total fungal particle release ($P = 0.0025$, $n = 10$). The effect of the air velocity and sampling time is shown in Figure 3, letters illustrate the statistical significant differences between the scenarios.

The release rate of fungal particles decreased over time (example in Figure 4). This was the case for both short- and long-term measurements as well as for both damp and dry surfaces. Little peaks can be spotted at the times where the P-FLEC was moved to a different location on the surfaces as described in the method section.

3.4. Potential particle deposition in the airways

There was no statistical difference between the D_g of the particles in the four release scenarios (Table 5). Calculations show that 90–91% of the particles released from the surfaces are within the inhalable fraction, 86–89% are within the thoracic fraction and 54–70% are within the respirable fraction. There were no statistical significant differences for the fractions of inhalable size, thoracic size, and respirable size between the four scenarios (Table 5).

4. Discussion

In general, the identified species from the infested gypsum boards were in agreement with what is typically found in damp buildings or on damp building materials. Of the 17 fungal species found in the EDC sampling from the moisture-damaged house, eight belong to group 1 of the ERMI index and five belonged to group 2. Six out of the 10 species recovered on the gypsum boards belonged to group 1 of the ERMI index and only two belonged to group 2 (Table 2). These results show that the laboratory simulation of a water-damage in a building was successful. Across all four release scenarios, a low richness in aerosols was discovered; in fact, only five of the 17 species in the inoculum were recovered in the aerosols generated from the infested gypsum boards. The low richness of aerosols is in agreement with a study by Niemeier et al. [28], who found only few species in FSSST (a device similar to the P-FLEC) samplings of surfaces with fungal growth in Finnish homes. In the present study, a reason for the low species richness might be due to the impact on the fungal growth when the gypsum boards were dried out before the surfaces were overgrown. Another reason could be the *Coniophora puteana*-like fungus influencing the release. Another study on the release of fungal aerosols [12] found, 12.0 and 9.1 species/m^2, in comparison, the present study found

Table 3
The total concentration of fungi over the four seasons, the M-ERMI value for each season as well as the GM of air change rate (ACR) and relative humidity (RH) measured over the four seasons in the five non-moisture-damaged homes (A-E).

Homes	Total concentration [CFU/m^2/day]	M-ERMI value Summer	M-ERMI value Autumn	M-ERMI value Winter	M-ERMI value Spring	ACR over seasons [16] [h^{-1}]	RH over seasons [%]
A	14,035	−3.71	4.88	4.40	0	0.4	48
B	17,544	−0.90	13.01	5.69	−6.60	0.8	55
C	16,746	−3.20	4.88	11.31	4.71	0.2	56
D	15,630	−4.45	12.39	5.18	7.39	0.2	53
E	18,979	−3.61	6.90	–	0.30	1.5	56
Estimate[a]	–	−9.8227	1.7683	–	−4.2898	–	–
P-value	–	0.0003	0.42		0.062	–	–

[a] Estimates and P-value of the effect of season on the M-ERMI value (Proc Mixed analyses).

Table 4
Concentration of the species found in each of the five non-water-damaged homes during each seasons.

	Concentration [CFU/m2/day]																			
	Summer					Autumn					Winter					Spring				
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
Aspergillus																				
A. flavus[a]									319											
A. fumigatus[a]	638																			
A. glaucus										159										
A. niger[a]																		319		
A. nidulans	638	638							638										159	
A. penicilloides[a]									159											
A. versicolor[a]							1595					319	319						319	
Candida																				
Ca. lambica[a]				638																
Ca. sorbosa[a]	638						159													
Chaetomium																				
Ch. globosum[a]										159										
Cladosporium																				
C. cladosporides[b]	638		638	2552	1276												159			
C. herbarum[b]	5104	5104	6380	7018	10,207					159										159
Epicoccum																				
E. nigrum[b]	638																			
Fusarium																				
F. proliferatum																	159			
Penicillium																				
P. brevicompactum[a]	638		2552	638	3190	159	797	478	319	319	159	478	159					159	478	
P. camemberti		638					159					2552	159	319			159			
P. chrysogenum[b]												797					1276			
P. citrinum														159						
P. corylophilum[a]										159			159							
P. glabrum[a]						478	319	159	478	159	159		159	478			319			319
P. olsonii						159	478													
P. roqueforti																159				159
Wallemia																				
W. sebi[a]							159		319										159	
Total	**12,759**	**7656**	**14,673**	**10,845**	**17,225**	**797**	**3668**	**638**	**2871**	**1116**	**319**	**4147**	**957**	**957**	**bd**	**159**	**2073**	**478**	**957**	**638**

The total CFU/m2/day for each home and season are including un-identified species that are not listed in the table. CFU= Colony Forming Units.
[a] Fungi frequently found in US homes and associated with mouldy buildings (ERMI Group 1).
[b] Fungi frequently found in US homes but not associated with mouldy buildings (ERMI Group 2).

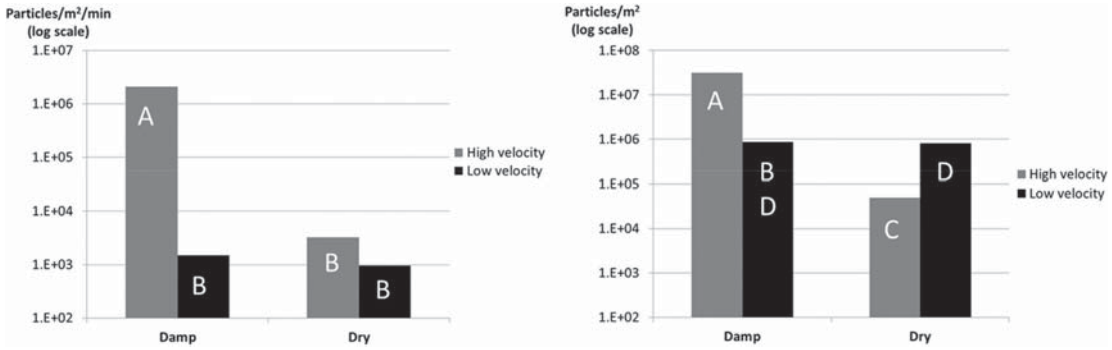

Fig. 3. The release rate [CFU/m^2/min] (left) and total release [CFU/m^2] (right) of fungal particles from both damp and dry surfaces. The release rate and release are shown for both high and low velocity over the surfaces. For the high velocity, the sampling time was 15 min, and for the low velocity the sampling time was 8 h. There is no statistical significance between columns with the same letters.

25.0 species/m^2. The study by Madsen et al. [12] was likewise conducted using EDC sampling of inoculum, P-FLEC for generating of aerosols, and MALDI-TOF for species identification.

The identification of viable fungi from the infested gypsum boards shows that *P. chrysogenum* and *A. versicolor* are by far the most frequently found species. This result is in agreement with the results of previous studies [22,23]. The study by Nielsen et al. [23] found that *P. chrysogenum* and *A. versicolor* are some of the most frequently present species on building materials, and that *P. chrysogenum* grows rapidly on gypsum boards. Likewise, the study by Gravesen et al. [22] found that *P. chrysogenum* and *A. versicolor* were found frequently on moisture-damaged building materials, especially on moisture-damaged gypsum boards. It is possible that *P. chrysogenum* and *A. versicolor* produce more spores

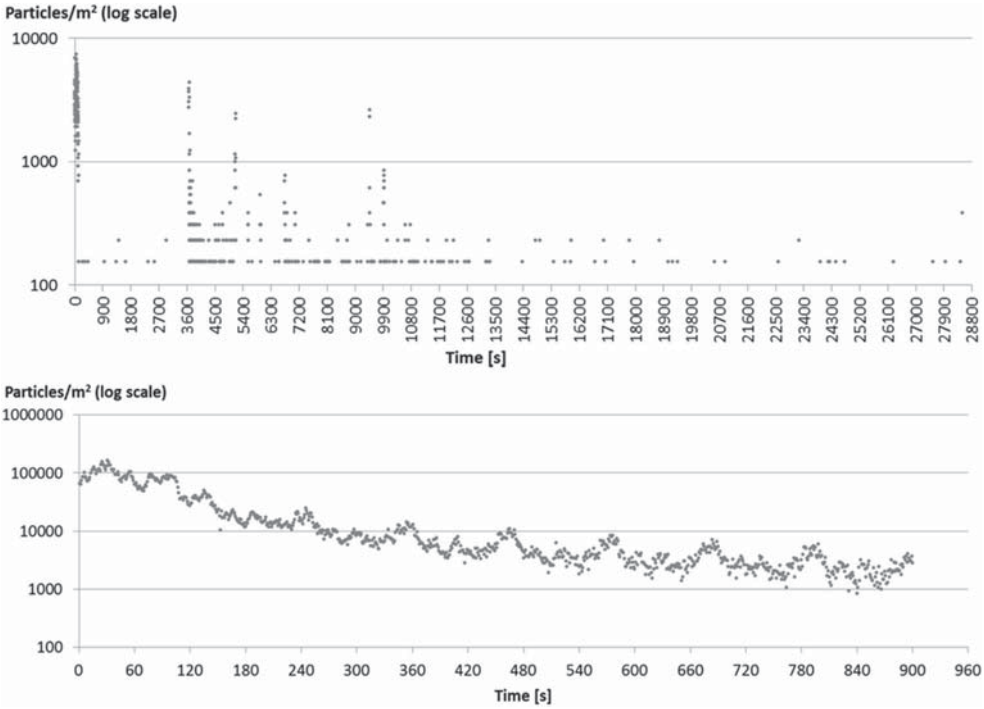

Fig. 4. Examples of concentrations of particles released as affected by low velocity (2.5 m/s) for 8 h over a dry surface (top), and as affected by a high velocity (9 m/s) for 15 min over a damp surface (bottom). Measured every second using the Aerodynamic Particle Sizer (APS).

Table 5
The calculated geometrical mean diameter (D_g), the standard deviation, and the potential deposition of particles in the respiratory system.

Scenarios	D_g [μm]	Geometrical Standard deviation	Inhalable fraction [%]	Thoracic fraction [%]	Respirable fraction [%]
1	3.1	2.5	90	89	54
2	3.3	2.0	91	86	69
3	3.5	2.5	91	88	70
4	2.6	2.1	91	87	68
P-value	0.95 n = 12	–	0.69 n = 11	0.63 n = 11	0.56 n = 11

than other species and therefore mask the other species in the aerosols. In the present study, the results of the MALDI-TOF identification and a visual inspection of the gypsum boards showed that *Ch. globosum* was growing on the surfaces of dry gypsum boards but was not recovered in the aerosols. A study of *Ch. globosum* growth on different media, describes that *Ch. globosum* produces spores in a slime head which makes the spores less likely to become airborne [32]. In spite of a fairly large amount of spores being inoculated on each board, it seems that only a few would dominate the individual boards. This indicate that using a multi fungal culture to conduct simulations of the exposure risk to fungal growth in buildings, might regardless result in surfaces colonised with only a few different spices. The fact that *S. chartarum* was only found on the damp surfaces was not unexpected since studies have found that *S. chartarum* needs not only a high RH but also elevated material moisture levels to proliferate on building materials [33].

The positive correlation between the ACR of the five non-moisture-damaged homes and the total level of viable fungi sampled with EDC is in accordance with the previous study, using GSP sampling, in the same homes [16]. However, the identification of fungal species from the five non-moisture-damaged homes, revealed a negative correlation between the measured ACR and M-ERMI values of the homes. This indicates that airing out will have a beneficial effect on the composition of the fungal species in the indoor air as measured by M-ERMI. The results also showed a positive correlation between the higher RH and higher M-ERMI values. In some cases, a high RH can lead to elevated moisture levels in the building materials or the finishing joints of the windows and thereby growth of fungi. Previous research has also found a positive correlation between high RH in buildings and concentrations of airborne fungi [14,34,35] as well as health symptoms amongst occupants [36]. The negative correlation between M-ERMI values and ACR and the positive correlation between the M-ERMI values and RH, is especially important in light of the health symptoms associated with living in homes with a high ERMI value [6–8]. The results also indicate that not only the material dampness, but also the RH of the indoor air is of importance when evaluating the occupants' exposure risk to airborne fungi. A previous study found that the RH was positively associated with the concentration of airborne fungi [35]. The significant increase found in the indoor fungal concentration during summer was due to high concentrations of two *Cladosporium* species. This is therefore likely due to the exchange of indoor and outdoor air [16,37,38], thus the outdoor fungal composition influences the indoor fungal composition as also suggested in other studies [39]. During summer season the

level of fungi in the outdoor air increases [16], and the general level of fungi in the indoor air consequently increases. The present study found a significant effect of season on M-ERMI values with lowest values in summer and highest in autumn and winter. This might be due to the differences in ACR and the outdoor composition of fungi across the seasons. This indicates that when using the ERMI value to evaluate the fungal exposure in a building, it is necessary to consider the seasonal variation. Further, the geographical region should be taken into account as indicated in a Finish study [40]. However, the Danish climate is temperate like the northern French climate and the climates of Boston and Detroit in the US, where the current ERMI Index has been used with success. On the other hand *P. chrysogenum* is in the ERMI categorised as a group 2 fungus [5], and in a similar index for Finland as a group 1 fungus [40], and this study indicates that the species might belong to group 1 as it was released from all the infested gypsum boards, but was only found in two home samples. Previous studies also found *P. chrysogenum* on moisture damaged building material [22,23]. Similarly, a study only found *P. chrysogenum* as a dominating species in 4 of 27 homes, but on all studied mouldy gypsum boards [12]. If *P. chrysogenum* was reclassified as a group 1 fungus this did not change the seasonal variation in M-ERMI, the negative association between M-ERMI and ACR nor the positive correlation between M-ERMI and RH (data not shown). Limitations of the ERMI index and the fact that is not a perfect tool for determining fungal growth in build environments has been discussed in the literature [7,40,41], e.g. a problem can occur if the log sum of group 2 fungi are low, in that case a more thorough investigation of the group 1 fungi must be considerate [41]. Further it is discussed that group 1 of the ERMI index is likely to be expanded with currently overseen species, as more research is conducted [7]. However there is still evidence that the ERMI index is a useful tool for evaluating the fungal burden of a building and has shown positive associations with asthma [9,11,41,42]. The strength of ERMI, as supposed to pervious established knowledge on fungal species in moisture damaged buildings, is that the ERMI includes identification on species level and not just genus [43–48].

The results of the present study indicate that it might also be possible to develop an index similar to the ERMI index based on a MALDI-TOF analysis. In fact, 25 of the 36 species in the ERMI index are present in a standard commercial available fungal MALDI-TOF MS database. Further, the data base used in the present study was equipped with two additional species from the ERMI index. On average it was possible to identify 94% of the species in the samples from the five non-moisture damaged homes. The results of the identification of the fungal species are important and were necessary to gain knowledge on the importance of ACR, RH, and season on the occupants' exposure risk to airborne fungi in buildings.

The increase of the release rate of fungal particles with increased air velocity near the damp surfaces means that an increase in the air movement near a surface with fungal growth might lead to an increased exposure risk to fungal aerosols. An increase in air movement could be caused by occupant's activities, such as airing out, cleaning, or cooking. The fact that the same result was not significant for the dry surfaces, might partly be due to the long time over which the study occurred, and to the variation in growth between the repetitions. The higher fungal particle release rate and total release from damp surfaces compared to dry surfaces was expected, mainly because the growth was stopped earlier on the dry surfaces than on the damp surfaces. Previous publications have studied the release of aerosols from gypsum boards totally covered by fungal growth and have found that a low RH will increase the fungal release rate [24,25]. This effect of RH has also been seen for *Penicillium* sp. and *A. fumigatus* on an agar medium [49]. In the present study, we have exposed the infested gypsum boards to higher air velocity or used a considerably longer timescale than in previous studies. This is expected to cause a higher fraction of the total spores to be released, which is in favour of a high release from the damp surfaces with most fungal growth. These studies together, show that the release rate from a totally fungal covered area is higher when it is dried out, but if the area potentially available for fungal growth is not totally covered by fungal growth, drying out will inhibit further growth and thus lower the spore release rate and total spore release. Therefore, fast action seems beneficial in a fungal remediation process, in order to reduce the occupants' exposure risk to airborne fungi.

An increased sampling time of fungal aerosols in exposure studies seem to be useful, since fungal particles were still released after 8 h with low velocity despite the fact that the release rate decrease over time. Further, the identification data showed higher species richness for the long-term sampling, where *A. fumigatus* and *A. flavus* were recovered in addition to *A. versicolor* and *P. chrysogenum*.

The lack of significant differences in the potential depositions of particles in the respiratory system between the four scenarios might be caused by the differences between the repetitions. A previous study [25] has found that the percentage of respirable fractions of fungal particles was higher during low RH. For the short-term sampling the same tendency was seen in the present study. The D_g of the aerosols in the present study was higher than what was found in a previous study [12]. This might be due to the differences in RH and air velocity near the infested surfaces as well as the species composition.

5. Conclusion

Increasing the ACR and lowering the RH will result in lower M-ERMI values which may result in a healthier species composition of the indoor air. More species were released after 8 h with a low air velocity than after 15 min with a high air velocity near the surface. Further, the air velocity near the surfaces in combination with varying sampling time had a significant influence on the release rate and total release of fungal particles. The higher richness of fungal species on the contact plates from the previously damp surfaces, the higher fungal particle release rate from damp surfaces with more substantial growth, and the fact that *S. chartarum* was only found on damp surfaces, together indicate that fast action by drying out areas with fungal growth in a building have a positive impact on the exposure risk for the occupants.

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Paper 3

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Inflammatory potential of low doses of airborne fungi from fungal infested damp and dry gypsum boards

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ABSTRACT

This study has investigated the total inflammatory potential (TIP) of low concentration fungal samples from moisture-damaged and fungal infested gypsum boards. The fungal aerosols were generated from damp and dried surfaces, and sampled using filter sampling and liquid impingement. The TIP of the samples was analysed using a granulocyte assay based on differentiated HL60 cells. The study found a tendency to a J-shaped dose-response curve for fungal samples. Low concentrations of fungi were aerosolised from the gypsum boards, and the aerosols were dominated by *Aspergillus versicolor* and *Penicillium chrysogenum*. *Bacillus infantis* and *Paenibacillus* sp. were found on the gypsum boards, but not recovered in the aerosols. A significant correlation was found between the TIP of diluted and undiluted samples of fungal aerosols. However, diluted samples had a higher TIP than undiluted samples, and no significant association was found between concentration of fungi and the TIP of the samples. This is likely due to the J-shaped dose response curve. The aerosol samples from the dried gypsum boards had a significantly higher TIP compared to aerosols from the damp surfaces. However, the J-shaped dose-response curve weakens the conclusion on the influence of surfaces dampness, sampling time, fungal species or sampling methods. It could, however, be concluded that samples from both damp and dry surfaces induce inflammation in the HL60 cells, despite the low concentration of fungi. Thus, a dried fungal infestation in a building seem still to present a concern for the occupant.

1. Introduction

Inflammation is an underlying cause of some health symptoms associated with exposure to fungi in damp buildings. Thus, *in vivo* as well as *in vitro* studies have found indications that exposure to fungi causes respiratory inflammation [1–5]. Review studies conclude that there is an association between dampness or fungal growth in buildings and symptoms amongst occupants such as, colds, fatigue, skin irritation, concentration difficulties, and even asthma and allergies [6–8]. Thus, studies have investigated factors that might influence the concentration of airborne fungi in indoor air [9–13], as well as the potential health effect of airborne fungi in indoor air, by investigating the inflammatory potential of home dust samples or aerosol samples with fungi in *in vitro* assays [10,14,15].

Environmental samples for investigation of the inflammatory potential of microbes in the indoor air seem to contain lower fungal concentrations (fungal spores or Colony Forming Units (CFU)/ml) than aerosol samples produced in the laboratory for investigation of the inflammatory potential. Thus, *in vitro* studies of fungal samples generated in laboratory settings have exposed cells to 10^5–10^7 CFU fungi/ml [10,14,15], while samples from the indoor air often contain $<10^2$ CFU (- or spores)/ml [11,16]. It is, however, difficult to relate a concentration in CFU/ml to a human indoor exposure. Typical concentrations found in buildings with visual fungal growth or moisture damage seem only rarely to exceed 10^4 CFU/m^3 [17]. Low fungal concentrations of the indoor air ($<10^3$ CFU/m^3 [17]) may occur if fungi are growing on hidden surfaces within the building structure, e.g. in between multilayer wall constructions or underneath a parquet floor. In these cases, the structure behind which the fungi are growing works as a barrier for the fungal particles [18,19]. In spite of low fungal concentrations in the indoor air, caused by a fungal infestation, occupants still seem to experience symptoms associated with fungal exposure [20]. However, knowledge on the inflammatory potential of low doses of fungi from infested building materials is limited.

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A way to assess an impact of fungal components on the human immune system is through sensitive *in vitro* studies using human cell lines like e.g. the HL 60 cell line [21]. The HL 60 cells are sensitive to microorganisms [14,22]. Thus, a study of aerosol samples from biofuel plants shows that four microbial factors (endotoxin, fungal spores, β-D-glucan, and actinomycetes) contributed to the total inflammatory potential (TIP) of the samples [14]. Likewise, a study of mixed fungal species aerosolised from infested gypsum boards used HL 60 cells to investigate the TIP of the fungal samples and found that the relative humidity (RH) during the incubation process influenced the TIP of the samples [10]. Studies on the effects of aerosols of mixed fungal species on the TIP have recently been suggested [13], partly based on the facts that several fungal genera are present on indoor surfaces [23] as well as in indoor air [13,24], and partly based on the lack of knowledge on the influence of different species and species combinations on the TIP. With the development of methods which quickly can identify fungal species correctly [25], it seems now to be possible to gain more knowledge on health effects of fungi on species level.

Different methods can be used to sample airborne microorganisms. One method, liquid impingement, has been suggested to capture mycotoxins [26] in addition to fungal particle, which likely could be of interest in regards to the TIP and toxicity of an aerosol sample. E.g. a study comparing filter sampling and liquid sampling found a higher TIP of the liquid samples, despite a lower concentration [27]. Sampling time may affect the species richness of a fungal aerosol sample [24], which again may affect the TIP of an aerosol sample. Likewise, studies on fungal spore release from gypsum boards have shown a difference in the number of fungal spores released from damp and dry surfaces, as well as in the TIP of the aerosol samples [10].

The aim of this study was to get knowledge on the TIP of low concentrations of fungi released from water-damaged fungal infested gypsum boards. The study is designed to get knowledge on how the TIP of a fungal sample is affected by the sampling time, sampling method, species composition, and by drying out the infested gypsum boards.

2. Method

2.1. Design and generation of fungal samples for the TIP investigation

The fungal aerosol samples were generated from water-damaged gypsum boards, inoculated with fungi sampled in a moisture-damaged house. Gypsum boards are sensitive to damp conditions and a commonly used material in buildings. Further, they contain cellulose which supports fungal growth [28] and have previously been used in investigations of fungal growth on building materials and spore release [10,12,29–31]. The gypsum boards were incubated at approximately 95% RH during the growth process. After 13 weeks, one half of the boards were dried and further incubated at 35–40% RH. A detailed description of the incubation and growth process and an illustration of the setup can be found in Ref. [24]. The P-FLEC (Particle-Field and Laboratory Emission Cell, Chematec, Denmark) was used for generation of the fungal aerosols. A bar with ten 0.8 mm nuzzles rotated 1 cm over the surfaces of the infested gypsum boards and scanned an area of 130 cm^2 during one rotation of 60 s. The air jets from the nozzle bar are directed towards the surface at an angle of 45°. In the chamber of the P-FLEC an airflow was created, and the released aerosols were thereby transported to the outlet at the top of the chamber. Here either a GSP (GesamtStaubProbenahme) (CIS by BGI, INC, USA) sampler or an impinger (BioSampler, SKC, UK) was connected to sample the aerosols (Fig. 1). Further, data on the particle size distribution measured by an aerodynamic particle sizer (APS) can be found in a previous study [24].

2.2. Sampling

Four aerosol sampling scenarios using both impinger and GSP sampling were performed with three repetitions of each of the aerosol sampling scenarios (Table 1, scenario 1–4). In addition, controls with gypsum boards without fungal growth were conducted for each aerosol sampling scenario. The gypsum boards for the control samplings were the same as the ones inoculated with fungi, but without moisture and fungal growth. During sampling with both impinger and GSP, the P-FLEC was moved slightly over the surfaces in regular intervals, every 2 min for the 15 min sampling (in the following referred to as short sampling time) and every 30 min for the 8 h sampling (in the following referred to as long sampling time). This was to ensure sampling over the entire surfaces of the gypsum boards (196 cm^2). The GSP and impinger samplings were conducted at individual gypsum boards. Both sampling time and air velocity near the surface were adjusted simultaneously to ensure that the boards were exposed to the same air volume across all four aerosol sampling scenarios. In addition to the aerosol samples, surface scrapings were conducted on a total of six damp and six dry surfaces. The surface scrapings were each conducted on individual boards after the aerosol samplings, but on the same boards as the aerosol samplings.

The impinger samples aerosols at a flow of 12.5 l/min into 20 ml impinger liquid (0.0005% Tween20 solution). The total sampling air volume was thus 187.5 l and 6000 l for the short- and long term sampling, respectively. The GSP samples at a flow of 3.5 l/min, and sampled the aerosols onto polycarbonate filters with pore size 1 μm (Frisenette, USA). The total sampling air volume was thus 52.5 l and 1680 l for the short- and long term sampling respectively. Surface samplings were done by gently scraping a sterilized scalpel over the entire surfaces of the gypsum boards (196 cm^2), making sure not to scrape the same area more than once.

2.3. Extraction and dilution of samples

The polycarbonate filters were extracted in 5.6 ml 0.0005% Tween20 solution to account for the differences in sampling volume between the GSP and impinger. The filter was set to shake at 300 rpm for 1 h. The scrapings were likewise suspended in 5.6 ml 0.0005% Tween20 solution.

A total of 24 aerosol samples (undiluted and 10× diluted) and 12 surface scrapings (undiluted and 10× diluted) were produced for investigation of the TIP (Table 1). The samples were diluted using HBSS (Hanks Balanced Salt Solution, Biological Industries, USA).

2.4. Plate dilution

The suspensions from the surface scrapings, the filter extractions, and the impinger sampling were plate diluted on DG18 ager plates (MERCK, Germany) for quantification and identification. The DG18 agar was chosen since it has been proven to be more effective than other agar media both in regards to quantity and species richness [32]. One sample from each of the four aerosol sampling scenarios (Table 1, scenario 1–4) and one sample from both scraping scenarios (Table 1, scenario 5–6) were in addition plate diluted onto nutrient agar (OXOID, UK) for quantification and identification of bacteria in the samples.

2.5. TIP investigation

The TIP of the fungal samples was measured using the

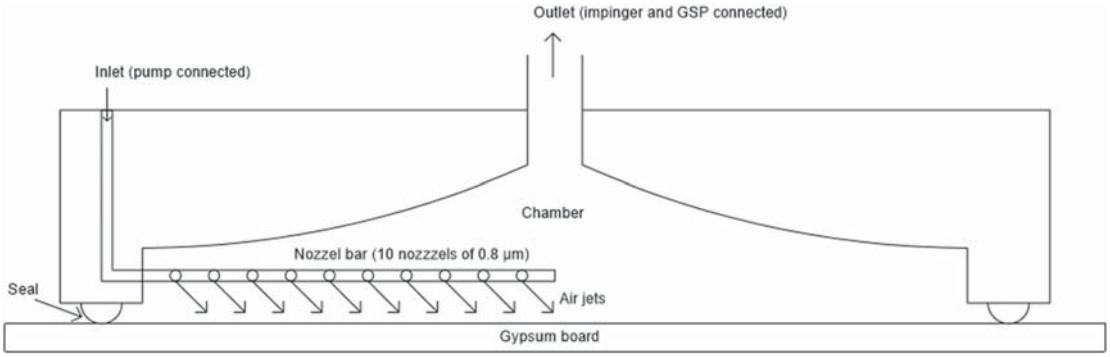

Fig. 1. Illustration of the P-FLEC used for generation of the fungal aerosols from the fungal infested gypsum boards. The nozzle bar is placed 1 cm above the surface of the gypsum board and has ten 0.8 µm nozzles, which via the pump produces the air jets that are directed towards the surface at an angle of 45°.

Table 1
Samples prepared for investigation of the total inflammatory potential. Listed are the sampling scenario for the dose-response analysis (0), the four aerosol sampling scenarios (1–4), and the two scraping scenarios (5–6). Both sampling time and air velocity near the surface were adjusted simultaneously to ensure the same sampling air volume across all four aerosol sampling scenarios.

Scenarios	Sampling type	Repetitions[b]	Sampling time	Air velocity	Growth medium	Incubation RH[c]
0	Impinger[a] and Scrape[a]	–	8 h	2.5 m/s	Malt extract agar	–
1	Impinger and GSP	3	15 min	9.0 m/s	Gypsum boards	Damp (>95%)
2	Impinger and GSP	3	8 h	2.5 m/s	Gypsum boards	Damp (>95%)
3	Impinger and GSP	3	15 min	9.0 m/s	Gypsum boards	Dry (35–40%)
4	Impinger and GSP	3	8 h	2.5 m/s	Gypsum boards	Dry (35–40%)
5	Scrape	6	–	–	Gypsum boards	Damp (>95%)
6	Scrape	6	–	–	Gypsum boards	Dry (35–40%)

[a] *Aspergillus fumigatus* and *Penicillium brevicompactum*
[b] The number of infested gypsum boards.
[c] The incubation RH after the initial incubation with a RH of 95%. Impinger = BioSampler SKC; GSP = GesamtStaubProbenahme, RH = Relative Humidity.

granulocyte assay, which is based on differentiated HL-60 cells (Human Promyelocytic Leukaemia cell line). Upon exposure to fungi, the differentiated HL60 cells react by producing reactive oxygen species (ROS) [22]. The cells were differentiated by adding All-trans Retinoic Acid (ATRA) and letting the cells differentiate for 6–7 days without changing the growth medium (RPMI 1640, Biological Industries, USA). The cells were seeded at 3×10^5 cell/ml and incubated at 5% CO_2 at 37 °C. A detailed description of the maintenance of the cells can be found in Timm et al. [14]. The reactive oxygen will react with luminol and through this reaction emit light that can be detected by a thermostated (37 °C) ORION II Microplate luminometer (Berthold Detection Systems, Germany), which measured relative-light units per second (RLU/s) every second throughout a period of 3 h. All samples were conducted in duplicates, and for each sample the RLU/s values were added and the average of the duplicates represents the TIP of the samples. On each Microplate a standards of endotoxin was included in dilutions of 0.25 EU/ml and 1.00 EU/ml as well as zymosan in dilutions of 1 µg/ml and 10 µg/ml. Further, controls in the form of HBSS and a 0.0005% Tween20 solution were included on each Microplate.

2.6. Dose-response relationship

The dose-response relationship was tested by producing fungal samples of two fungal species, *Aspergillus fumigatus* and *Penicillium brevicompactum*, in high concentration (Table 1, scenario 0). This was done by growing the fungi on malt extract ager (OXOID, UK) in large petri dishes (D = 15 cm). For both fungal species, the P-FLEC in connection with the impinger was used for aerosol sampling. The aerosol samplings were conducted over a period of 8 h, and with an air velocity of 2.5 m/s near the surfaces. After the aerosol samplings, a scrape of *P. brevicompactum* was made. The concentration of the samples was estimated by spore count in a microscope using a Haemocytometer (Bright Line, Hausser Scientific, USA). The TIP of the three samples (two aerosol samples and one surface scraping) was measured using the granulocyte assay. Three dilutions of each sample were used to create the dose-response curve. The samples were diluted using HBSS. In contrast to the aerosol samplings and other investigations of the present study, these samples were not conducted in repetitions.

2.7. Background ROS of fungal samples

The fungal samples were tested for production of ROS themselves which might influence the measured ROS level of the samples in the TIP investigation. This was done by conducting the granulocyte assay without HL60 cells. Background ROS was tested for a total of eight aerosol samples and four scrapings and for both diluted and undiluted samples (Table 2). After testing the selected samples without HL60 cells, four aerosol samples and six scrapings were further tested with dead HL60 cells (apoptotic cell death) to test if a difference occurred using dead HL60 cell as opposed to no HL60 cells, in the background ROS testing. The apoptotic cell death was obtained by leaving the cells in HBSS at 4 °C for five days. The cells were checked by microscopy regularly, to ensure that the desired result in regards to apoptotic cell death (dark shrunken cells) was achieved. Table 2 provide an overview of the samples tested without HL60 cells and with dead HL60 cells.

Table 2
Samples used to test if the fungal samples produced ROS themselves. Listed is the number of tested samples, the dilutions levels, and if the samples have been analysed with dead[a] HL60 cell or without HL60 cells.

Samples	No. of samples	Dilutions	Dead HL60 cells[a]	No HL60 cells
HBSS control	–	–	+	+
Impinger sample, damp surfaces (Scenario 1 and 2)	4	Undiluted and 10× diluted[b]	+	+
Impinger sample, dry surfaces (Scenario 3 and 4)	4	Undiluted and 10× diluted[b]		+
Scraping, damp surface (Scenario 5)	2	Undiluted and 10× diluted[b]		+
Scraping, dry surface (Scenario 6)	2	Undiluted and 10× diluted[b]		+
Scraping, *Penicillium brevicompactum* (scenario 0)	6	$10\times - 10^6\times$ diluted[b]	+	+

[a]Apoptotic cell death.
[b]Samples were diluted using HBSS (Hanks Balanced Salt Solution, Biological Industries, USA).

2.8. Analysis of fungal toxicity

Duplicates of one of each aerosol sample were tested for toxicity by conducting a dead and viable cell count after 40 min and 90 min of exposure to the fungal samples using the granulocyte assay. This was done on a NucleoCounter NC-100™ (Chemometec, Denmark) which is used for total cell count, and discrimination between viable and non-viable mammalian cells. An amount of 50 μl of the cell suspension was loaded into the Nucleo Cassette, and the NucleoCounter counted non-viable cells in the suspension. By adding Lysis buffer and Stabilizing buffer in the ratio 1:1, the total cell count was obtained. Subtracting the two provided the viable cell count of each suspension [33].

2.9. MALDI-TOF MS for identification

For identification of both fungi and bacteria, the MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization-Time-Of-Flight) analysis was performed on an Autoflex III (Bruker Daltonics, Germany). The ethanol extraction method was used for identification of fungi. A detailed description of the process can be found in Ref. [24]. For identification of bacteria, the formic acid method was applied. A small part of the bacteria was spotted onto a steel target plate (Bruker Daltonics, Bremen, Germany), and 1 μl formic acid (SIGMA-ALDRICH, USA) was applied to each sample. After the formic acid had dried, 1 μl Matrix (Bruker Daltonics, Germany) was applied and allowed to dry before analyse. The Bruker Biotyper 3.1 software with the BDAL filamentous library 1.0 was used for analysis.

2.10. Data treatment

Statistical analyses were made in the Statistical Analysis Software (SAS 9.4), and all values were log transformed for analyses. The Proc CORR command was used to investigate the correlations between fungal concentrations and the TIP of the fungal samples, between the TIP of the undiluted and 10× diluted fungal samples, and between the concentration of the fungal species *Aspergillus versicolor* and *Penicillium chrysogenum*. The Proc ANNOVA command was used for analyses of differences in the TIP and concentration of the samples in regards to dampness, sampling time, and sampling device (GSP vs. impinger), and for analysis of the difference in toxicity between undiluted and 10× diluted samples. The paired T-test analysis was applied to check for statistical difference between the data point of the dose-response curves. The PROC MIXED analysis was used to compare differences in the concentration of the species.

3. Results

3.1. Background ROS and dose-response curves

The fungi in the samples did not produce any ROS themselves (example in Fig. 2).

Analyses of the dose-response relationship showed a tendency to a J-shaped dose-response curve of the 100× diluted, 10× diluted, and undiluted aerosol samples of *Aspergillus fumigatus* and *Penicillium brevicompactum* (Fig. 3, left). The concentration of the undiluted *A. fumigatus* aerosol sample was 2.1×10^6 spores/ml, and the concentration of the undiluted *P. brevicompactum* aerosol sample was 8.0×10^4 spores/ml. Likewise did the dose-response curve from the scrapings of *P. brevicompactum* with respectively 10^5X diluted, 10^4X diluted, and 100× diluted samples show a tendency to a J-shaped curve (Fig. 3, right). The concentration of the undiluted sample of the surface scraping from *P. brevicompactum* was 1.5×10^8 spores/ml. A paired *t*-test analysis showed no statistical difference in the measured TIP for the two lowest concentrations of the dose-response curves ($p = 0.15$). For the two highest concentrations of the dose-response curves a statistical difference in the measured TIP was found ($p = 0.02$).

3.2. Species composition of fungi and bacteria in the samples

In total, nine fungal species and two bacterial species were found in the aerosol and surface samples (Table 3). Controls for each aerosol sampling scenario were conducted, and in all cases no CFU of fungi were recovered. Four of the nine fungal species were found in the GSP samples, and three of the nine fungal species were found in the impinger samples, while five were found in the scrapings. *Penicillium chrysogenum* and *Aspergillus versicolor* were dominating the samples in terms of concentration and were also found most frequently amongst the samples (Table 3). The concentrations of *P. chrysogenum* and *A. versicolor* were negatively correlated ($r = -0.43$, $P = 0.01$, $n = 24$). The concentration of *A. versicolor* tended to be higher in aerosols from damp than from dry gypsum boards ($p = 0.060$). Opposite, concentrations of *P. chrysogenum* tended to be higher in aerosols from dry gypsum boards ($p = 0.11$).

Stachybotrys chartarum was found on the surfaces of the damp gypsum boards by visual inspection and microscopy (Table 3) but was not recovered on the DG18 agar plates.

Bacteria were found in the scrapings of both damp and dry surfaces but were not recovered amongst the aerosols. *Bacillus infantis* and *Paenibacillus* sp. were found on the dry surfaces, while *Bacillus infantis* was found on the damp surfaces (Table 3).

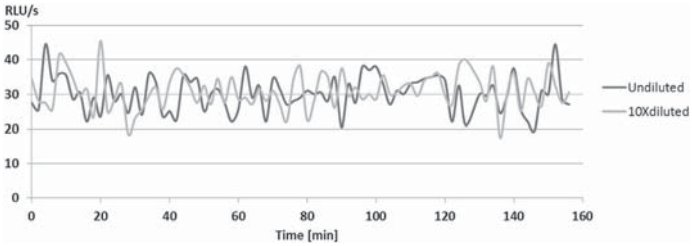

Fig. 2. Example of the background ROS production measured as RLU (Relative-light Units) of an impinger sampling with low velocity over a damp surface, as function of time.

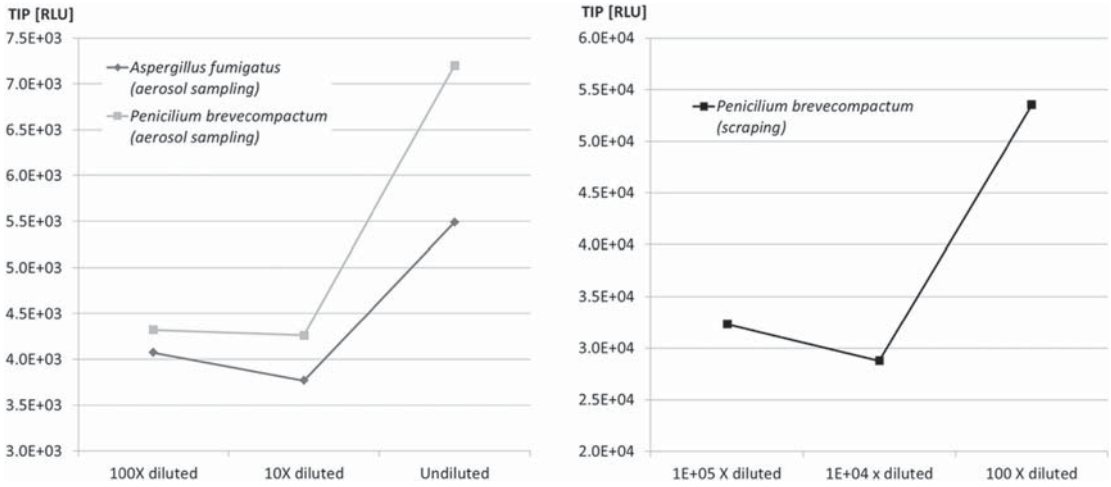

Fig. 3. Doses-response curves for *Penicillium brevicompactum* and *Aspergillus fumigatus* cultivated on Malt extract agar. Left diagram for aerosols from respectively *A. fumigatus* (undiluted 2.1 × ^{10}6 spores/ml) and *P. bervicompactum* (undiluted 8.0 × 10^4 spores/ml). Right diagram for scrapings from *P. brevicompactum* (1.5 × 10^8 spores/ml).

The impinger tended to sample a larger fungi CFU/m^2 compared to the GSP sampler for the damp surfaces ($p = 0.14$), while the GSP tended to sample a higher fungi CFU/m^2 compared to the impinger for the dry surfaces ($p = 0.30$). For the damp surfaces, the tending difference in sampled fungi CFU/m^2 between impinger and GSP was largest for the scenario with long sampling time (Fig. 4).

3.3. TIP

For all samples, the TIP of the undiluted samples was lower than the TIP of the 10× diluted samples ($p = 0.0005$) (Fig. 5, left), and a positive correlation was found between the TIP of the undiluted and 10× diluted samples ($r = 0.651$, $P = 0.0005$, $n = 24$). For the impinger samples, the TIP of the undiluted samples was below the detection limit (example in Fig. 5, right). For these reasons only, the TIP of the 10× diluted samples is used for analysis in the following. Thus in the following, when referred to the TIP of a sample, it is the TIP of the 10× diluted samples.

There was no significant correlation between the fungal concentration of the samples and the TIP of the samples ($r = 0.137$, $P = 0.42$, $n = 24$). A significant difference in the TIP for impinger versus GSP samples from the dry surfaces was found ($P < 0.0001$) (Fig. 6). However, for the damp surfaces the tendency was a higher TIP of the impinger samples than of the GSP samples (Fig. 6). Likewise, a significant difference in TIP of samples from dry versus damp surfaces was found ($P < 0.0001$), with dry surfaces resulting in the highest TIP, and long sampling time by impinger being an exception (Fig. 6).

3.4. Toxicity

The percentage of viable cells after exposure to the fungal samples for 40 min shows that most cells are in fact still viable (Fig. 7). After 90 min of exposure to the fungal samples, the results were similar with no remarkable increase in cell death (2–12% cell death). Further, there was no significant difference between undiluted and 10× diluted samples ($p = 0.27$, $n = 3$) (Fig. 7).

4. Discussion

This study shows no linear dose-response relationship between the TIP of a fungal aerosol sample and the concentration of fungi in the sample. The dose-response curves for both the scrapings and the two aerosol samples showed a tendency to a J-shaped curve. Thus, samples with low concentrations had a higher TIP than the less diluted samples, and samples with highest concentrations had the highest TIP (Fig. 3). The concentrations of fungi in samples from the gypsum boards are rather low (10^2–10^5 CFU/ml; GM = 1.43 × 10^4 CFU/ml), and seem only to show the first part of this J-shaped dose-response curve, where lower concentrations

Table 3
The species composition and concentrations (CFU/ml) of fungi in the impinger, GSP, and scraping samples. Data is shown for damp and dry surfaces as well as for long and short sampling times. For the total concentration, the Geometrical Mean (GM) of the repetitions is shown. For the individual species, the maximum concentration is shown.

	Damp					Dry				
	GSP		Impinger		Surface	GSP		Impinger		Surface
	Short sampling time	Long sampling time	Short sampling time	Long sampling time	Scrape	Short sampling time	Long sampling time	Short sampling time	Long sampling time	Scrape
Aspergillus										
A. flavus							2.3×10^3			
A. fumigatus		1.0×10^3								
A. niger								5.0×10^3		
A. ustus					0.002					
A. versicolor	5.9×10^5	1.15×10^5	1.4×10^4	8.7×10^4	3.8×10^6	0.256		3.0×10^3		9.1×10^5
Chaetomium										
Ch. globosum										2.0×10^3
Penicillium										
P. chrysogenum	9.1×10^3	807	2.1×10^4	5.0×10^3	1.5×10^6	5.4×10^4	5.1×10^4	2.0×10^3	2.9×10^4	8.5×10^5
P. glabrum										1.3×10^6
Stachybotrys										
S. chartarum	U	U	U	U	U					
Total fungi	$\mathbf{1.15 \times 10^4}$	$\mathbf{1.05 \times 10^4}$	$\mathbf{8.37 \times 10^3}$	$\mathbf{2.31 \times 10^4}$	$\mathbf{5.92 \times 10^5}$	$\mathbf{8.58 \times 10^3}$	$\mathbf{1.15 \times 10^4}$	**965**	**713**	$\mathbf{6.86 \times 10^5}$
S*[b]	**1.15**	**2.33**	**1.47**	**1.56**	**2.10**	**1.91**	**1.49**	**1.80**	**2.41**	**1.98**
Bacillus[a]										
B. infantis					25					722
Paenibacillus sp.[a]										120

a Gram-positive bacteria.
b The multiplicative standard deviation (S*) is calculated as described by Limpert et al. [34].
U = Unknown concentration (only discovered by visual Inspection of the surfaces), CFU=Colony Forming Units.

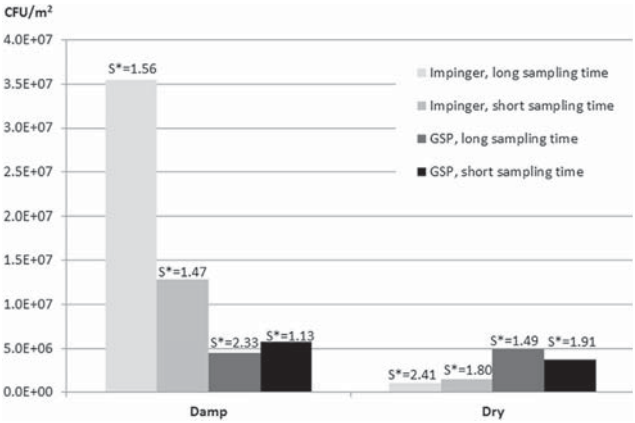

Fig. 4. Fungi CFU/m^2 sampled by impinger and GSP for both damp and dry surfaces as well as for long- (8 h) and short term (15 min) sampling. The CFU/m^2 values are all geometrical means of the three repetitions. The multiplicative standard deviation (S*) is calculated as described by Limpert et al. [34].

result in a higher TIP. It is therefore important to bear in mind the possibility of a J-shaped dose-response curve when measuring the TIP of a sample with a low concentration of fungi, since the result might otherwise be wrongly interpreted. Toxicology studies, both *in vitro* and *in vivo*, have found J-shaped or U-shaped dose-response curves (often just referred to as U-shaped) for a number of different agents, such as PCB, alcohol, and a general wide range of chemical agents [35–40]. However, to our knowledge there are no publications on this phenomenon using low doses of fungi as inflammatory agents in the HL 60 cell line.

The lack of correlation between the fungal concentration and the TIP of the samples is in accordance with other studies using low concentrations of fungi in environmental samples [11,16]. This lack of association between the TIP and fungal concentrations of the samples may be because of the tendency to a J-shaped dose-response curve, or it may be assumed that not only the fungal concentration plays a role in regards to the TIP of a fungal aerosol sample. The presence of a specific fungal species, metabolites, mycotoxins, or the combinations of fungi might influence the TIP of a fungal sample, and this might be more pronounced if the concentration is low. The aerosols in this study were all dominated by *P. chrysogenum* and *A. versicolor*, which concentrations were negatively correlated. In spite of that, it is not possible to conclude how much the single species contribute to the TIP of the samples in the present study. Likewise, it is assumed that the scrapings would include large numbers of hyphal fragments, and that this would

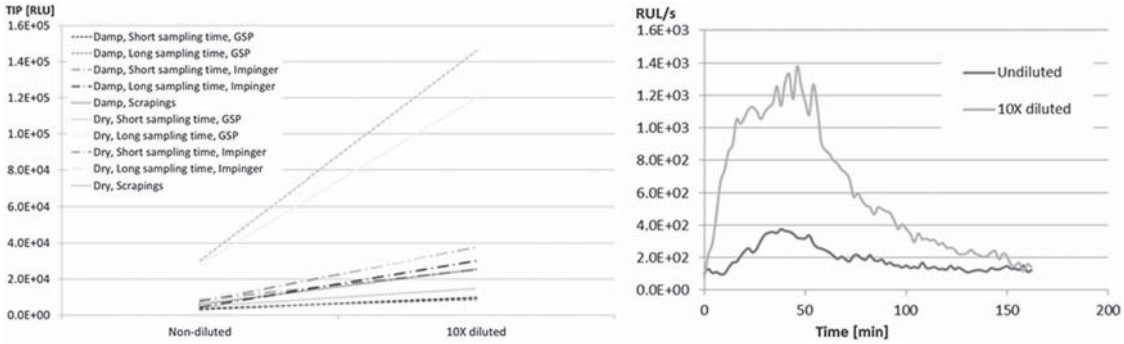

Fig. 5. Left, graphic illustration of the TIP relationship between the undiluted and 10× diluted fungal samples. Right, an example of the development of RLU over time of an undiluted and a 10× diluted fungal sample. The example is from a surface scraping of a damp surface and demonstrates the general picture for all fungal samples.

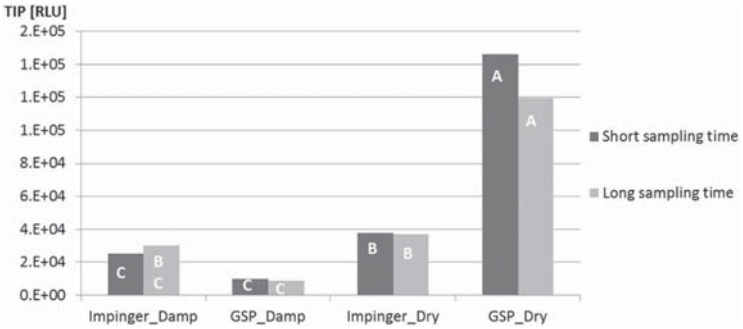

Fig. 6. The Total Inflammatory Potential (TIP) of the GSP and impinger samples from damp and dry surfaces and with long- and short sampling times. There is no statistical significant difference between bars with the same letter ($p < 0.0001$).

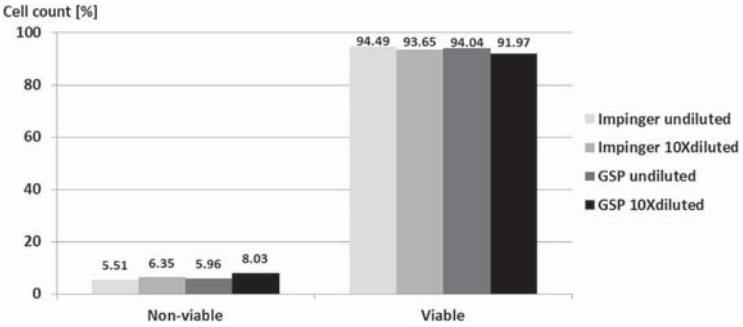

Fig. 7. Percentage of viable and non-viable granulocyte like cells after exposure to 10× diluted and undiluted impinger and GSP samples with fungi. Cells were exposed to the fungi for 40 min.

result in a higher TIP of the surface samples, but this was not seen. Studies with higher concentrations of fungi than what were used in the present study found a positive significant association between fungal concentration and the TIP of the samples [10,14]. This could be because the studies were done with concentrations of fungi in the more linear part of the dose-response curve.

The toxicity of the fungi in the samples was tested to ensure that the low TIP of the undiluted samples was not in fact due to toxicity of the components in the samples. There was no indication that this should be the case, in fact 92–94% of the cells were viable after 40 min and 90 min of exposing the granulocytes like cells to the fungal suspensions. It should, however, be borne in mind that cell death due to the toxicity of a sample might happen over a longer period so conducting toxicity analyses after 24 h might be necessary.

Some plant pathogenic fungi produce ROS when attacking plant

host cells [41], and as an attempt to test if there was a ROS production of the fungal samples themselves, the ROS production of the fungal samples both without the granulocyte like cells and with dead cells (apoptotic cell death) was studied. This test showed no ROS production from the fungi themselves, and thus the measured ROS seems only to be produced by HL60 cells.

Fungal aerosol samples from the dry surfaces had higher TIP than fungal aerosol samples from damp surfaces, which is in accordance with a similar study [10]. Another study showed that the respirable fraction of fungal particles was larger when fungi were aerosolised from gypsum boards with low RH compared to boards with high RH [12]. Together this indicates that a dry fungal damage may be of concern for the occupants. Unfortunately, the J-shaped dose-response curve of the low concentration fungal samples make it difficult to conclude how factors like sampling time, dampness, and species composition might influence the TIP of the fungal samples.

Even if no statistically difference was found, there was still a tendency towards the impinger sampling a higher amount of cultivable fungi compared to the GSP over damp surfaces, and that the reversed is the case for the dry surfaces. The fact that the tending difference was more pronounced for the scenario with long sampling time over damp surfaces indicates that some viable fungal parts from the damp surface may lose viability in the dry environment of the GSP sampler. This hypothesis is supported by a study that has found sensitive microorganism to be better recovered by liquid impingement than filter sampling [42]. The least robust fungal spores on the dry gypsum boards might already have lost their viability during the drying process, or the spores may have developed a protecting layer as e.g. melanin [43,44] during the drying process, thus their survival is not affected by the sampling on a filter. Measurements in homes show that higher concentrations of fungi, already exposed to environmental stress, are sampled in higher concentrations with the GSP compared the impinger [27], which is in accordance with the tendency seen in this study for dry gypsum boards. In addition to affecting the survivability, there might be a difference in the sampling efficiency of the GSP and impinger.

Penicillium chrysogenum and *Aspergillus versicolor* were the two most dominating fungal species in the samples, which is in accordance with other studies that find *P. chrysogenum* and *A. versicolor* to be the most frequently found fungal species on water-damaged building materials [45–47]. *P. chrysogenum* tended to be found in the highest concentrations in aerosols from dry surfaces and *A. versicolor* tended to be found in the highest concentrations in the aerosols from damp surfaces. A reason for this might be that *P. chrysogenum* grows rapidly on gypsum boards [46] and therefore was better established on the boards by the time they were dried out. Bacteria were found in surface samples of both dry and damp surfaces but were not recovered amongst the aerosols. It is important to be aware of the presence of bacteria on fungal infested surfaces in the context to a remediation process of a fungal- or moisture-damaged building. Even though some bacteria are not easily aerosolised, they might spread if the fungal infested material is not carefully handled during the remediation process. Previous studies have showed bacteria to contribute to the TIP of an aerosol sample [11,14,15,48].

5. Conclusion

The fungal aerosol samples from the fungal infested gypsum boards contained low concentrations of fungi. The aerosols did not affect the viability of the HL60 cells in the studied period, but in spite of a low concentration they induced an inflammation in the HL60 cells. The inflammatory response was seen from samples released from damp as well as from dried gypsum boards, and in GSP as well as in impinger samples. Thus, it can be concluded that a dried fungal infestation in buildings seem still to be of concern for the occupants.

The tendency to a J-shaped dose-response curve, and the fact that diluted samples had a higher TIP than undiluted samples, weakens the conclusion about the influence on TIP from sampling method, sampling time, dampness of the gypsum boards, or the presence of a specific fungal species. More research on factors that may influence the TIP of samples with low fungal concentrations is therefore necessary. Further, bacteria were present on the surfaces of the fungal infested gypsum boards, however they seem not to be aerosolised, but should never the less be taken into consideration, for example during a remediation process.

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