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Norwegian Building Research Institute

Climate2000

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Report from the R&D-programme «Climate 2000»

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Preface

This report presents a literature review. Its focus is to give an overview of known methods in measuring mould, how to evaluate the results of the measurements, and finally how to handle and prevent a mould problem. The work has been carried out as part of project 12 *Weather protection in the construction process. Critical Decisions – Causes and Consequences – Protective Actions* within the Norwegian research and development programme *Climate 2000 - Building constructions in a more severe climate*.

The Climate 2000 programme's principal objectives are to develop solutions in principal for building structures resulting in both increased durability and reliability in the face of external climatic impact, and to survey the possible impacts of climate change on the built environment. The intention is to define more accurate criteria and Codes of Practice for the design and construction of critical elements of building envelopes. Climate 2000 is an important part of the continuous development of the Building Research Design Sheets in the SINTEF Building Research Series, and product documentation in the form of technical approval and certification.

The programme is being managed by SINTEF Building and Infrastructure and carried out in co-operation with the Norwegian Defence Estates Agency, the Research Council of Norway (NFR), the Norwegian State Housing Bank, Norway's Directorate of Public Construction and Property (Statsbygg), the Norwegian Financial Services Association (FNH), National Office of Building Technology and Administration (BE), the Norwegian University of Science and Technology (NTNU) and a large number of key players in the construction industry. The programme was initiated in August 2000, and will continue until the end of 2007.

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Trondheim, October 2006

Tore Kvande
Programme manager
SINTEF Building and Infrastructure

Summary

This report gives an overview of different measuring- and analyzing methods to moulds, and how to evaluate these results compared to a possible mould problem indoor. The report also gives an overview of some guidelines of how to deal with and prevent mould growth indoor.

Sampling strategy is an essential part of the exposure assessment. According to general guides, the sampling strategy should maximize the probability of true-positive and minimize the possibility of false-negative findings. However, a choice of sampling techniques depends on the actual purpose of measurements, and no single method may be appropriate for the extensive identification of fungal growth and fungal exposure assessment. Therefore, air sampling as well as samples of settled dust, surface and biologically contaminated bulk materials or fluids are recommended and used for environmental monitoring.

Environmental monitoring is often based on the determination of culturable or total spore concentrations in samples possibly combined with the identification of fungi, more often on the generic than species level. However, little is known about the inhalation exposure to possible causative agents of fungi that may be responsible for a large variety of health effects observed in the epidemiological studies. During the recent years, more attention has been paid to the development and applications of analytical methods for fungal components and products, e.g. for (1-3)-β-D-glucan, ergosterol, mycotoxins, microbial VOC, allergens, extracellular polysaccharides and their use in the fungal exposure assessment.

Fungal and other microbial material is present on nearly all indoor surfaces. There is a great deal of uncertainty and variability in samples taken from indoor air and surfaces, and it may be difficult to discern which organisms are part of the natural background and which are the result of problematic contamination. However, the information gained from a careful and complete survey may aid in the evaluation of contamination sources and remediation needs.

The most effective way to manage mould in a building is to eliminate or limit the conditions that foster its establishment and growth. Every organism has strategies for locating a hospitable environment in obtaining water and nutrients, and reproducing. Intervention in one or more of those strategies can improve the resistance of the environment against microbial contamination

Contents

1 Introduction

Indoor dampness may be associated with some respiratory health effects, and a causal role for micro organisms has been suggested. However the specific roles of infectious and non-infectious micro organisms and their components in diseases related to indoor environments are poorly understood. The lack of knowledge regarding the role of micro organisms in the development and exacerbation of those diseases is due largely to the lack of valid quantitative exposure-assessment methods and knowledge of which specific microbial agents may primarily account for the presumed health effects. In most studies, exposure is assessed by means of questionnaires, and relatively few studies have attempted to measure exposure to micro organisms (Institute of Medicine, 2004).

Indoor environment contains a complex mixture of live (viable) and dead (nonviable) micro organisms, fragments thereof, toxins, allergens, microbial volatile organic compounds (MVOCs), and other chemicals. Sensitive and specific methods are available for the quantification of some biologic agents, such as endotoxins, but not for others. Many of the newly developed methods – for example, measurement of microbial agents, such as $\beta(1\rightarrow3)$-glucans or fungal extracellular polysaccharides (EPSs) – have not been well validated and are not commercially available. Even for some well-established methods, such as the Limulus amebocyte lysate (LAL) assay for measuring bacterial endotoxins, substantial variation in exposure assessment between laboratories has been demonstrated. It is known that the conditions of storage and transport of bioaerosol samples and extraction of dust samples may affect the activity of some biological agents, such as endotoxins, and thus their measured concentrations, but those conditions are not often addressed. Finally, there may be biological agents whose health effects have not been identified. Microbial exposure assessment in the indoor environment is therefore associated with large uncertainties, which potentially result in large measurement errors and biased exposure-response relationships (Institute of Medicine, 2004).

This report gives an overview of different measuring- and analyzing methods to moulds, and how to evaluate these results compared to a possible mould problem indoor. The report also gives an overview of some guidelines of how to deal with and prevent mould growth indoor.

2 Moisture and microbial measurements

Dampness and other excess moisture accumulation in buildings are closely connected to observations of mould, mildew, or other microbial growth. The behaviour of moisture and air movements can be characterized with physical parameters, but the biological phenomena take place according to a complicated network of regulating factors. Several phenomena make up the microbial ecology of an indoor environment (Institute of Medicine, 2004).

In principle, common saprophytic environmental micro organisms and their spores are present everywhere and they start to grow wherever their basic needs for growth are met. They differ enormously in their needs for environmental conditions and some fungi or bacteria always do well in practically any indoor micro environmental conditions. Many environmental micro organisms easily start growing on any surface that becomes wet or moistened. The minimal moisture need for microbial growth may be characterized in terms of the water activity of the substrate, a_w, which is the ratio of the moisture content of the material in question to the moisture content of the same material when it is saturated. In a situation where the material is in equilibrium with surrounding air that has a RH of 100%, $a_w = 1$ (Institute of Medicine, 2004).

The lowest aw at which the most tolerant, so-called xerophilic fungi may grow is 0.7, which correspond to an RH of 70%. Most fungi and bacteria require nearly saturated conditions; that is, a_w of at least 0.85-0.90 (Grant et al., 1989).

Along the life span of a building, weather changes and other events often cause at least temporary wetting of some its parts. Signs of microbial growth can thus be detected on many parts of a structure. Airborne spores and cells also accumulate in the parts of the structure that are in contact with soil or outdoor air, especially parts that act as sites of infiltration of intake air. Accumulated spores may or may not grow in these sites, depending primarily on moisture condition (Institute of Medicine, 2004).

The time it takes for fungi to grow on a particular material depends on the material's characteristics, the fungal species, and the amount of moisture. Moulds are also capable of producing large quantities of spores within a short time. Rautiala et al. (1996) reports massive fungal growth within a week after fire fighting efforts (Rautiala, Nevalainen and Kalliokoski, 2002). According to Pasanen et al. (1992a), a fungus can grow and sporulate within a day in moist conditions and within a week on occasionally wet indoor surfaces. With a RH above 80 % for several weeks or months, mould can grow in wood when the temperature is 5-50 °C. At RH above 95%, mould can be seen within a few days (Viitanen, 1997). In wetted gypsum board inoculated with spores, fungal growth started within 1-2 weeks (Murtoniemi et al., 2001). Chang et al. report a latent period of 3 days for fungal growth on ceiling titles, during which the germination and mould growth could be arrested (Chang, Foarde and Vanosdell, 1995).

Besides water, micro organisms need proper nutrients and temperatures to grow; some also need particular light conditions. Those circumstances are usually met in buildings. Even if modern building materials do not appear to be readily biodegradable, they may support microbial action (Institute of Medicine, 2004).

Microbial nutrition's may be carbohydrates, proteins, lipids and other biologic molecules and complexes, or they may be nonbiologic compounds. Nutrients are provided by house dust and available moisture and by many surface and construction materials, such as wallpapers, textiles, wood, paints, and glues. Even non biodegradable material, such as ceramic tiles and concrete, may support microbial growth (Hyvärinen, 2002) by providing a surface for

colonies. That explain why fungal colonies may be found on mineral fibre insulation – a material that would not seem hospitable to microbial growth (Walinder et al., 2001; Hyvärinen, 2002).

Prevailing temperatures in living spaces and other sections of buildings are usually 0-55 °C, that is greater than freezing and less than the temperature at which the denaturalisation of proteins would start. That range permits the growth of most environmental micro organisms even if the temperature is not optimal for a particular genus or species. Many environmental microorgansims are not especially strict in their temperature demands, in contrast with many pathogenic micro organisms that need the human body temperature to be able to grow (Institute of Medicine, 2004).

Time is another integral element in the assessment of microbial growth in buildings. Growth may be slowed by decreasing or increasing temperatures or other limiting factors, and the time window that must be considered in building microbiology is weeks, months or even years. It is known that microbial degradation normally consist of a chain of events, in which different groups of micro organisms follow each other (Grant et al., 1989), but present knowledge of building microbial ecology does not allow accurate estimation of the age of microbial damage on the basis of the particular fungal or bacterial flora observed.

3 Selection of sampling type

3.1 Bulk Sampling

Bulk samples are portions of environmental materials (e.g., settled dust, section of wallboard, pieces of duct lining, carpet segments, or return-air filters) tested to determine if they may contain or be contaminated with biological agents. The objective of such sampling is to collect a portion of material small enough to be transported conveniently and handled easily in the laboratory while still representing the material being sampled. Testing is done to determine if organisms (e.g., micro organisms or dust mites) have colonized the material and are actively growing as well as to identify surfaces where previously airborne biological particles have deposited and accumulated (ACGIH, 1999)

In general, bulk samples are cut or otherwise aseptically removed from a source and placed in clean, new or sterilized containers. Suitable containers for bulk samples are sterile jars for dry items or sterile bottles for water samples. New paper bags may be adequate to transport dry material samples. Sealable plastic bags are useful for samples of ventilation duct lining, ceiling tiles, wallpaper, and similar materials. To preserve the integrity of samples and avoid cross contamination, paper bags may be placed in plastic bags with a packet of desiccant material to keep the sample dry. The amount or volume of sample to collect and the manner in which to remove and transport it depend on the sample type and the analytical methods to be applied (ACGIH, 1999).

Samples of loose materials (e.g., carpet dust for antigen detection) can often be conveniently collected using a suction device (ACGIH, 1999).

3.2 Surface Sampling

3.2.1 General

Surface sampling during IEQ investigations is frequently linked to bulk and air sampling. Surface samples can provide information similar to that obtained from bulk samples regarding whether environmental materials may be contaminated beyond background levels and possibly serve as sources of biological agents that may be disseminated as bioaerosols.

Surface sampling may be used to;

a) confirm the nature of suspected microbial growth on environmental surfaces,
b) measure the relative degree of biological contamination,
c) and identify the types of micro organisms and other biological agents present.

Surface sampling is preferred over bulk sampling when a less destructive method of sample collection is desired (ACGIH, 1999).

Surface samples are collected by removing material;

a) with a suction device,
b) by pressing a collection material (e.g., a contact plate or adhesive tape) onto a surface,
c) or by washing a prescribed area with a wetted swab, cheesecloth or gauze swatch, or filter.

As compared to bulk dust sampling from floors, upholstered furniture, or other porous or fabric surfaces, the amount of material removed for a surface sample is generally small and the surface tested are generally smooth (ACGIH, 1999).

3.2.2 Contact sampling

Loose particles may be collected by pressing a contact plate to a surface, or applying an adhesive material to lift off sample material

3.2.3 Agar Contact Sampling

Contact plates are special culture dishes or flexible containers with a meniscus of agar extract beyond the container's rim. Advantages of the contact-plate method are that it is fairly easy to conduct (e.g., no filter or pumps are needed), and the exposed plates are simply shipped overnight to a laboratory for incubation and examination. Disadvantages of this method are the limitation inherent in all culture-based analyses as well as the possibility that growth on a contact plate may be so heavy that counting and identification of the isolated micro organisms is impossible (ACGIH, 1999).

3.2.4 Adhesive Tape Sampling

If information about viable micro organisms on environmental surfaces is not needed, the adhesive-tape method can provide information on the types and relative concentrations of biological particles that are present. Such samples can be collected using clear adhesive tape or packing tape or commercially available sampling strips. For microscopic examination of collected particles, adhesive tapes must bee of good optical quality and compatible with any strains the analytical laboratory may use on the specimens (ACGIH, 1999).

Adhesive-tape samples for examination by microscope are simple to collect. Many samples can be collected in a short amount of time; the results do not depend on the culturability of collected micro organisms; and samples that show hyphal fragments and reproductive structures can provide evidence of microbial growth, not just the presence of settled spores. However, the value of the information obtained depends on the field investigators´ decisions on where to sample. Usually several fungi contribute to visible growth, and multiple samples from such areas may be necessary to accurately assess the kinds of fungi present. Tape sampling is not quantitative and does not yield information on the extent or degree of environmental contamination. Analyst unfamiliar with environmental tape samples may find them difficult to read because environmental samples often contain extraneous material not present in tape samples prepared from laboratory cultures. When possible, investigators should also collect scrapings of material suspected of being microbial growth so that a laboratory analyst can prepare specimens of the material in other ways for examination by microscope (ACGIH, 1999).

3.2.5 Surface-Wash Sampling

In the surface-wash method, a swab, filter, or cheesecloth or gauze swatch is used to wipe a specified surface area. The collection media may be wetted with sterile water or wash solution to enhance particle solution. Samples for culture-based analysis must be handled aseptically for example, by using sterile forceps or touching only the bare end of a swab stick. A swab can be used to inoculate a culture plate immediately, or swabs, filters, and swatches can be shipped to a laboratory for analyses. Samples may be transferred to a laboratory dry in individual sterile containers or in a test tube with a sterile transport medium. Wipe samples can be processed similarly to dust samples (ACGIH, 1999).

3.3 Air sampling

Airborne biological material may consist of (ACGIH, 1999);

1. individual micro organisms, spores, or pollen grains,
2. aggregates of micro organisms, spores, pollen, or other biological material,
3. product or fragments of micro organisms, plants, arthropods, birds or mammals,
4. or any of the above carried on particles.

There are three standard methods of active sampling of airborne bioaerosols.

- *Impactor methods*. With impactor sampling, bioaerosols moving in the air stream pass through a round jet or a slit to a culture medium, adhesive microscope slide or tape strip. Applications of the principle of inertial impaction are seen in slit samplers, single-stage and multiple-stage impactors, centrifugal samplers and liquid impingers.
- *Liquid impinger methods*. Liquid impingers collect micro organisms by directing the air stream into a liquid collection fluid. Bacteria, viruses, and fungal spores are retained in the collection fluid and can subsequently be plated onto appropriate culture media or evaluated with other analytical techniques, although some re-entrainment and losses occur.
- *Air filtration methods*. Several sampling methods in common use rely on filtration to collect bioaerosols from a sampled air volume. After sampling, filters are agitated or sonicated in a solution. The solution is then serially diluted and plated on culture media or examined with analytical techniques

The most common volumetric samplers are summarized in table 1. When air samples are chosen for monitoring, several aspects should be considered: Representativeness of sampling, sampler performance, and possibilities to conduct various analyses. It is well-known that the shorter a sampling time is, the larger is the variability between side-by-samplers, and the lower is the representativeness of sampling (Pasanen, 2001).

Table 1
Commonly used sampler types for airborne fungi (Pasanen, 2001)

Sampler	Principle	Example of devices	Sampling Capacity	Possible analyses
Impactors and Sieve samplers: ♦ Spore traps ♦ Slit Samplers ♦ Cascade impactors	Impaction on ♦ Agar ♦ Sticky surface ♦ Glass slide ♦ Membranes	♦ Burkard ♦ Rotorod sampler ♦ Andersen impactor ♦ SAS ♦ Casella impactor ♦ May impactor ♦ Sierra Marple impactor	If volumetric: ♦ Air flow rate: 2 – 180 L/min ♦ Sampling time: from minutes to hours, up to a week	♦ Cultivation ♦ Microscopic analyses
Impingers	♦ Impaction ♦ Centrifugal force ♦ Diffusion into the liquid	♦ Shipe sampler ♦ AGI- 30 ♦ Midget, multi stage and micro-impingers	♦ Air flow rate: 0.1 – 55 L/min ♦ Sampling time: from minutes to hours	♦ Cultivation ♦ Microscopic analyses ♦ Biochemical analyses ♦ Immunoassays
Centrifugal samplers	Centrifugal force into ♦ Liquid ♦ Semi-solid ♦ Agar	♦ RCS ♦ Aerojet cyclone	♦ Air flow rate: 40–1000 L/min ♦ Sampling time: from minutes to hours	♦ Cultivation ♦ Microscopic analyses ♦ Biochemical analyses ♦ Immunoassays
Filter cassette	♦ Inertial impaction ♦ Interception ♦ Sieving onto fibrous, flat or membrane filters	♦ Glass fibre ♦ Cellulose ester ♦ Polycarbonate ♦ Teflon filters	♦ Air flow rate: 1–1000 L/min ♦ Sampling time: hours	♦ Cultivation ♦ Microscopic analyses ♦ Biochemical analyses ♦ Immunoassays

4 Sampling strategy

Sampling strategy is an essential part of the exposure assessment. According to general guides, the sampling strategy should maximize the probability of true-positive and minimize the possibility of false-negative findings. However, a choice of sampling techniques depends on the actual purpose of measurements, and no single method may be appropriate for the extensive identification of fungal growth and fungal exposure assessment. Therefore, air sampling as well as samples of settled dust, surface and biologically contaminated bulk materials or fluids are recommended and used for environmental monitoring (Dillon et al., 1996). In addition, the exposure assessment may also be based on biomonitoring with serum or urine samples of exposed people (Biagini, 1999; Bünger et al., 1999). Those techniques are commonly used in industrial and occupational settings, but less frequently applied to indoor environments.

Surface and bulk samples with different sampling techniques (swabs, tapes, contact plates) and preparation procedures (dilution and direct plating) are commonly used in the detection of fungal contamination in buildings (hazard identification) and verifying remedial measures (Crook, 1996; Dillon et al., 1999). Instead, settled dust (house dust) samples have been taken to assess fungal exposure because of easy replicate sampling and because they are assumed to represent a long-term exposure better than short-duration air sampling (Dillon et al., 1999; Flannigan, 1997; Verhoeff and Burge, 1997). However, distinct differences in the composition of fungal species and the percentage of culturable spores have been shown between dust and air samples collected in the same interior space. In addition, the capability of spores to become airborne is known to vary between different fungal species. These facts hamper the interpretation of results from dust samples and reduce the usefulness of dust sampling for the exposure assessment (Flannigan, 1997; Verhoeff and Burge, 1997; Dillon et al., 1999). Air sampling is the most widely used sampling technique in the fungal exposure assessment. The simplest method for fungal sampling is gravimetric/settles plates, though it is no longer recommended because of many defects (Pasanen, 2001).

In the literature, a lot of comparison data is available on the performance and sampling efficiency of different samplers and on the use of different culture media (Verhoeff et al., 1990; Nevalainen et al., 1992; Jensen, 1995; Cage et al., 1996). The limit of detection and the upper limit of range are often neglected when the sampling techniques and procedures are considered. However, those factors have a significant impact on the reliability of results. In addition, it should be noted that the sampling technique used may limit the possibilities for further analyses. Many sampling devices are designed for the determination of only culturable fungi or for total spore counting (Table 1). Filter sampling, certain impingers, spore traps and cyclones enables also other analyses, e.g. biochemical analyses and immunoassays (Zwick et al., 1991; Crook, 1996).

5 Assessment methods

5.1 Introduction

Environmental monitoring is often based on the determination of culturable or total spore concentrations in samples possibly combined with the identification of fungi, more often on the generic than species level (Dillon et al., 1999) However, little is known about the inhalation exposure to possible causative agents of fungi that may be responsible for a large variety of health effects observed in the epidemiological studies. During the recent years, more attention has been paid to the development and applications of analytical methods for fungal components and products, e.g. for (1-3)-β-D-glucan, ergosterol, mycotoxins, microbial VOC, allergens, extracellular polysaccharides and their use in the fungal exposure assessment.

5.2 Culture-based methods

Airborne exposure to micro organisms in the environment can be studied by counting culturable propagules in air samples (or in settled dust samples). After sample collection colonies of bacteria and fungi are grown on culture media at a defined temperature over a 3-7 day period. Colonies are counted manually or with the aid of image analysis techniques (Douwes et al., 2003).

Counting of culturable micro organisms has some serious drawbacks including poor reproducibility, selection for certain species due to chosen culture media, temperature etc. and the fact that dead micro organisms, cell debris and microbial components are not detected, while they too may have toxic and/or allergenic properties. In addition, good methods for personal air sampling of culturable micro organisms are not available, and air sampling for a period of more than 15 min is often not possible, whereas air concentration usually vary largely in time. On the other hand, counting of culturable micro organisms is potentially a very sensitive technique and many different species can be identified. Traditionally used culture methods have proven to be of limited use for quantitative exposure assessment. Culture-based techniques thus usually provide qualitative rather than quantitative data that can, however, be important in risk assessment, since not all fungal and bacterial species pose the same hazard (Douwes et al., 2003). An extensive review on techniques for sampling and culturing micro organisms are published by Eduard and Heederik (Eduard and Heederik, 1998).

5.3 Non-culture methods

Non-culture-based methods enumerate organisms without regard to viability. Sampling of non-culturable bioaerosols is generally based on air filtration or liquid impinger methods. Micro organisms can be stained with a fluorochrome, e.g. acridine orange, and counted with a epifluorescence microscope (Thorne et al., 1994) Possibilities of classifying micro organisms taxonomically are limited because little structure can be observed. Electron microscopy (EM) or scanning EM can also be used and allow better determination (Eduard and Aalen, 1988; Karlsson and Malmberg, 1989) Simple light microscopy may be used to count micro organisms, but counting is based only on morphological recognition, which may result in severe measurement errors. The main advantage of microscopy is that both dead and living micro organisms are quantified, selection effects are limited, personal air sampling is possible and sampling time can be varied over a large range. Disadvantages include laborious and complicated procedures, high costs per sample, unknown validity, no detection of possibly relevant toxic or allergenic components or cell debris, while possibilities for the determination of micro organisms for most of these techniques are limited (Eduard and Heederik, 1998).

5.4 Microbial constituents

Instead of counting culturable or non-culturable propagules, constituents or metabolites of mould can be measured as an estimate of exposure. Toxic (e.g. mycotoxins) components can be measured but also non-toxic molecules may serve as markers of either large groups of microorganis or of specific microbial genera or species. The use of advanced methods, such as polymerase chain reaction (PCR)-based technologies and immunoassays, has opened new avenues for detection and speciation regardless of whether the organisms are culturable. Some markers for the assessment of fungal biomass include ergosterol measured by gas chromatography–mass spectrometry (GC-MS) (Miller and Young, 1997) or fungal extracellular polysaccharides measured with specific enzyme immunoassays (Douwes et al., 1999) allowing partial identification of the mould genera present.

Ergosterol is found in the membrane of a majority of fungi but is absent from most other micro organisms (Schnurer, 1993). Measurement of ergosterol is difficult to perform and therefore is not performed by most analytic laboratories.

Measurement of dust allergens tests hypotheses about the amount of exposure individuals have to fungal allergens irrespective of whether sources of fungal contamination are still present. An occupant might be exposed to fungi allergens in dust sufficient to cause symptoms, even when few or no airborne spores are present. The characterized method for measuring house dust allergens is with EIAs for specific allergens or for allergenic species (Rogers, 2003). Commercial assays for fungal allergens (Alt a 1 and Asp f 1) are available, as well as assays for whole fungal species (Chew et al., 2003; Barnes et al., 2001). Because fungi are known to have varied expression of allergens, the absence of specific proteins does not necessarily prove the absence of those fungi. Polyclonal antibody–based assays detect a broader range of fungal antigens; however, they might correlate poorly with the presence of spores. Portnoy et.al have demonstrated that airborne spore counts and dust antigen assays of fungal allergens in dust correlate for certain species, such as *Cladosporium and Aspergillus* (Portnoy, Barnes and Kennedy, 2004), but they do not correlate well for others, such as *Alternaria* species (Barnes et al, 2001.). Polyclonal assays are useful to document the removal of sources of allergenic material (Arbes et al., 2003).

A discrepancy between culturable and total spore counting led to efforts to find better estimates of fungal biomass resulting in applications of the cell wall component measurements (Dillon et al., 1999; Miller, Dales and White, 1999; Rylander, 1999) From those, β(1→3)-glucan has been considered to be most appropriate, because they are potent activators of numerous cells, such as macrophages and neutrophils, in the human immune system (Rylander and Holt, 1998; Rylander and Lin, 2000). Beta-1,3-D glucans are straight or branched chain glucose polymers that are present in most fungi either bound to chitin or on the cell wall as free polymers (Ruiz-Herrera, 1991). Two methods to measure β(1→3)-glucans have been described, one of which is based on the Limulus amoebocyte lysate (LAL) assay (Aketagawa et al., 1993) and the other on an enzyme immunoassay (Douwes et al., 1996).

Volatile organic compounds produced by fungi may be suitable markers of fungal growth. Fungi produce a complex mixture of low-molecular weight and high-molecular-weight volatile compounds. Many of these MVOCs have extremely low odour thresholds (1-10 ppt), causing a musty smell that might be noticed by the occupant before significant problems develop (Elke et al., 1999). More than 500 different MVOCs have been identified, including mixtures of alcohols, aldehydes, amines, aromatic and chlorinated hydrocarbons, ketones, sulfurbased compounds, and terpenes. Measurement of MVOCs can be performed to test hypotheses related to the presence of fungi as a source of substances that are aesthetically unpleasant. In most cases MVOCs do not by themselves cause health problems, acting instead as irritants (Fischer and Dott, 2003), however, most persons find the smell to be

repulsive. MVOC measurement can be used to identify hidden sources of fungal contamination. Sampling methods generally involve capture onto an adsorbent material, followed by desorption and detection in the analytic laboratory. It is also possible to capture a small volume of air in an evacuated container for subsequent injection into the gas chromatography mass spectroscope for chemical analysis. The level of detection decreases as the volume of air sampled increases. Attempts to develop species-specific MVOC profiles have met with some success (Fischer et al., 1999), although the sampling and analytic procedures are too expensive for routine use at this time. Limitations to MVOC analysis are that a single sample represents one point in time that might not represent typical conditions. The actual concentration of MVOCs might vary by orders of magnitude depending on ventilation, substrate moisture levels, availability of food sources, and competing micro organisms. In addition, some volatile organic compounds might originate from nonmicrobial sources (Fischer and Dott, 2003). Several new technologies might address these limitations. A portable gas chromatography and/or gas chromatography mass spectroscopy (Inficon, Syracuse, NY) unit now can be carried from room to room, with a sampling probe allowing for real-time gas sampling. With this, the investigator could track MVOCs to source locations. MVOC analysis with these devices can be performed in as little as 3 minutes, although the level of detection generally is not as low as for analyses of larger volumes of air. The zNose (Electronic Sensor Technology, Newbury Park, Calif) is a portable, ultra fast gas chromatography analyzer with a quartz crystal–based acoustic wave interferometer detector that is used to create a reproducible 360° pattern or Vaporprint. Prism Analytical Technologies (PATI, Mt Pleasant, Mich) has developed an ultrahigh sensitivity method by using a specially designed sampler containing multiple matrices. The MOLDSCAN sampler can detect concentrations as low as 300 ppt.

Many genera of fungi have evolved the ability to produce toxic metabolites (Jarvis and Miller, 2005). Their purpose is to inhibit the growth of competing organisms. The list of known mycotoxins is extensive and ranges from relatively simple sesquiterpenes, such as lemonine, to complex heterocycles, such as cyclosporine. Since their implication in animal diseases in the 1960s, mycotoxins have been the subject of intense scientific interest (Harrach et al., 1983). Public interest in mycotoxins has increased because of concerns over biologic warfare, the T-2 toxin, and toxins from *Stachybotrys* species (Jarvis, 2003). Mycotoxins are present in relatively small concentration on individual spores, and many species produce mycotoxins with similar structures, making analysis difficult. Identification and measurement of mycotoxins require advanced analytic instrumentation, such as gas chromatography mass spectroscopy or liquid chromatography mass spectroscopy (Lagana et al., 2003). Building materials grossly contaminated with fungi, such as *Stachybotrys* species, might produce sufficient quantities of mycotoxin to be measured (Flappan et al., 1999; Hodgson et al., 1998), and they have been detected in urine of exposed human subjects (Croft et al., 2002). Samples for mycotoxin analysis can be collected from contaminated materials, such as drywall, carpet, or wood, or even from house dust.

Finally, PCR techniques have been developed for the identification and quantisation of specific species of bacteria and fungi in the air (Alvarez et al., 1994; Khan and Cerniglia, 1994). PCR allows amplification of small quantities of target DNA, typically by 10^6–10^{10} times, to determine in a qualitative manner the presence of specific micro organisms. Application of quantitative PCR for analysis of air samples containing micro organisms is still under development but is expected to find applications in situations where specific infectious micro organisms may be present (Douwes et al., 2003).

Most of the methods to measure mould constituents are in an experimental phase and have as yet not been routinely applied and/or are not commercially available. Important advantages of these methods include:

i) the stability of most of the measured components, allowing longer sampling times for airborne measurements, and frozen storage of samples prior to analysis;
ii) the use of standards in most of these methods;
iii) the enhanced possibility to test for reproducibility.

Pasanen (2001) have made an overview of the advantages and disadvantages of a selection of methods (Table 2 – 4).

Table 2
Analytical methods for fungal propagules (Pasanen, 2001)

Fungal agent	Methods	Advantages	Disadvantages
Culturable fungi	Cultivation on different media and incubation conditions	♦ Enables species identification ♦ Not require special equipment ♦ Large reference data available	♦ Always selective ♦ Underestimate total counts ♦ Overestimate tolerant species ♦ No consensus on recommended media ♦ Slow method
Specific fungal species, e.g. toxigenic or pathogenic species	♦ Cultivation ♦ Immunochemical methods ♦ Chemical methods ♦ Molecular biological techniques (PCR)	♦ Indicate moisture problems ♦ Causative agents of health effects ♦ Some techniques (e.g. PCR and immunochemical) are specific, fast, sensitive, independent of viability	♦ Require expertise or special equipment ♦ Cultivation: slow and selective ♦ E.g. PCR is semi quantitative, costly ♦ Most techniques are available for only certain species
Total spores	♦ Microscopy: ♦ Epi-fluorescence ♦ Bright-field, phase contrast light microscope ♦ Scanning electron microscope ♦ Image analyzer	♦ Independent of viability ♦ Fast, basically automated ♦ Some techniques do not require special, expensive equipment	♦ Background matrix disturbs ♦ Overestimate large, pigmented spores ♦ Require expertise or special equipment ♦ No information on species

Table 3
Analytical methods for fungal cell wall components (Pasanen, 2001)

Fungal agent	Methods	Advantages	Disadvantages
Ergosterol	♦ HPLC ♦ GC-MS ♦ GC-MSMS	♦ A good estimate of fungal biomass ♦ Specific, fast, high reducibility ♦ Enables analysis of a large series of samples at a time	♦ May not be sensitive for air samples ♦ Little reference data ♦ No information on fungal species ♦ May underestimate yeasts
Extracellular polysaccharides	Immunochemical method (ELISA)	♦ Independent of viability ♦ No special, costly equipment ♦ Fast	♦ No reference data ♦ Indicator value not known yet ♦ No commercially available
(1→3)-β-D-glucan	♦ Limulus amoebocyte lysate method (LAL) and its modifications ♦ Immunochemical (EIA)	♦ Possible causative agent ♦ Independent of viability ♦ EIA reproducible, less expensive, possibly more specific than LAL ♦ LAL commercially available, highly sensitive	♦ No information on species ♦ LAL not highly specific ♦ EIA not sensitive for indoor air samples, no commercially available ♦ Little reference data

Table 4
Analytical methods for fungal metabolites and allergens (Pasanen, 2001)

Fungal agent	Methods	Advantages	Disadvantages
Mycotoxins	♦ Screening tests: ♦ Cell culture test ♦ Bioassay ♦ Mycotoxin analyses ♦ Chemical methods (HPLC, TLC, HPLC-MSMS, GC-MS, FTIR) ♦ Immunochemical tests	♦ Causative agents ♦ Screening tests: ♦ Fast and low-cost ♦ Immunochemical tests: ♦ No special equipment ♦ Simple sample preparation ♦ High specificity ♦ Chemical method: ♦ Sensitive	♦ Screening tests: ♦ Allow a possibility of false-positive results ♦ Chemical methods: ♦ Costly ♦ Complex sample preparation ♦ Special equipment ♦ Immunochemical tests: ♦ Not highly sensitive ♦ No methods for indoor air samples
Volatile metabolites	Sampling into carbonbased or TENAX adsorbents Analysis by GC-FID, Thermal desorption GC-MS, GC-MSMS	♦ Indicates moisture and odour problems ♦ Fast method	♦ Indicate active growth phase ♦ Unclear indicators: no consensus on relevant MVOC ♦ Difficulties with interpretation ♦ Little reference data
Allergens and specific antibodies	Immunochemical methods (EIA, RIA)	♦ Causative agents ♦ IgG antibodies indicate a long term exposure ♦ No special equipment ♦ Simple sample preparation	♦ A few well-known fungal allergens ♦ Poor availability of good-quality reagents ♦ Difficulties with e.g. ♦ Cross-reactivity

6 Result evaluation

6.1 General

There are no numerical health based criteria for airborne fungi for the general indoor environment. The lack of agreement in the industrial hygiene/public health community off what instrument(s) to use to collect airborne fungi (or what fungal particulates to collect), coupled with the variety of reporting formats, underlies a fundamental circuitous problem. There are no health based standards because of the lack of dose response data, there is no dose response data because there are no standardized sampling protocols, and there are no standardized sampling protocols due to the lack of standards. As a result, bioaerosol data requires interpretation for assessing the building environment (Dillon, Heihnson and Miller, 1996; Eduard and Heederik, 1998; Fung and Hughson, 2003). As with sampling protocols, a standard data evaluation format for bioaerosols is also lacking, and building assessment guidelines in general are based on consensus rather than objective scientific criteria. A standard data evaluation model often used is based on assessing differences in "biodiversity" between two sample sets of interest (Dillon, Heihnson and Miller, 1996; ACGIH, 1999; Macher, 1999).

The concept of "biodiversity" has its roots in the field of ecology, and the context, derivation, and applicability of biodiversity as used in traditional ecology is relevant in a discussion regarding bioaerosol data evaluation. Ecologists recognize several different aspects of biodiversity, most often for the purpose of assessing the effects of pollution on a naturally occurring community. A particular ecological study may define biodiversity by a variety of criteria to include the number of species (species richness) and/or various abundance measures (i.e., population counts, biomass). Further, geographical/spatial distribution of organisms are implicit in the structure of ecological communities, which in turn dictates sampling and assessment criteria, and ultimately characterization of the organisms and communities under consideration (Magurran, 1988). As a result, the array of variables that can be used to describe and define biodiversity are frequently combined and ultimately obscured, and none of the myriad of ecological diversity indices that have been developed is universally accepted or applicable. The inherent subjectivity of biodiversity has resulted in ecological diversity studies that are often not replicable, and the lack of clear and consistent definition and quantification has resulted in considerable disagreement among ecologists as to the scientific usefulness of the term (Magurran, 1988; Ludwig and Reynolds, 1988a; Ludwig and Reynolds, 1988b). It should also be recognized that the concept of biodiversity for bioaerosol data interpretation and building investigation is applied in a fundamentally different way and from a different perspective than the parent ecological concept from which it has been derived. A strict ecological approach for fungal bioaerosols in a building would evaluate the factors that alter the overall fungal species makeup in a given building habitat relative to a reference zone such as the outdoor air. However, the indoor environment of any operating building is constructed with many building materials that are microbial nutrient sources, and with a myriad of potential niches artificially maintained (either by design or through building failure) at optimum growth conditions for fungi. Since an operating building is by definition "different" from the general ambient environment, a difference in the overall species profile (one definition of fungal biodiversity) in the indoor air would be expected even in a "healthy" building, and would not necessarily be relevant to public health and building diagnostic issues that bioaerosol data attempts to determine.

Rank order analysis of fungal types has historically been cited as one approach in evaluating biodiversity of airborne fungi. When applied to building evaluation, the underlying logic is that two environments of interest are similar if there is no significant difference in the rankings of total airborne concentration of each fungal type (Dillon, Heihnson and Miller,

1996; Macher, 1999; Eudey, Su and Burge, 1995). In the case of comparison of a suspect building and the general outdoor air, common outdoor fungi such as *Cladosporium* and *Alternaria* will still very likely be the most highly ranked in a problematic indoor environment, even when problematic species (i.e., *Aspergillus*, *Penicillium*) are amplified over their normally low levels in the outdoor air. That is, the detection in several samples indoors (at low concentration) of fungi that are normally detected infrequently in the outdoor air, often will not exhibit a difference in rankings between the indoor and outdoor environments. Accordingly, quantifying differences in rankings using Spearman's rank correlation, an otherwise very powerful statistic, results in false positives and false negatives. Differences in rankings that may appear to represent a significant difference between two test fungal populations often occur coincidentally, which limits rank order analysis as an objective evaluative tool (Macher, 1999; Spicer and Gangloff, 2000).

Some investigators do not perform a genus variability assessment where the total count is low (e.g., less than 100 CFU/m³). A low colony count is subject to considerable variability. The collection of only a few spores in a small air volume can vary a lot due to sampling and analytical errors (Hess-Kosa, 2002).

The assessment may also be limited by laboratory reporting techniques. Most laboratories identify the more prevalent genera, up to a limited number (e.g., five of the most numerous mould colonies). Some identify all recognizable genera on the basis of growth structure and patterns, whereas others identify the most prevalent genera. Many will attempt to identify species of *Aspergillus* as well (e.g., *Aspergillus flavus*). Other genera must be re-plated for species determination. This involves more expense and culturing time (e.g., an additional 2 weeks). A colony growth may be declared as unidentifiable, or it is referred to as mycelia sterilia. The latter means the mould is sterile, does not for fruiting bodies/spores, in the nutrient medium provided. These (mycelia) can, however, be replated onto other media where they may grow and potentially be identified. Due to recent concerns, most laboratories will also identify one of the species of *Stachybotrys* (e.g., *Stachybotrys chartarum*) (Hess-Kosa, 2002).

Other approaches to include evaluation of differences in total spore levels (regardless of species), differences in the total of combined *Aspergillus* and *Penicillium* species, differences in most frequently detected fungi, and differences in total number of fungal species detected have been used in various studies (Duchaine, Grimard and Cormier, 2000; Kemp et al., 2003; Lappalainen, Lindroos and Reijula, 1999; Meklin et al., 2003; Shelton et al., 2002). As with ecological diversity, the lack of clear definition and consistent quantification underscores the subjectivity and limitations of directly applying biodiversity models for building microbial investigations (Hess-Kosa, 2002).

6.2 Fungal concentrations and flora

Concentrations of viable airborne fungi vary between 10^1-10^5 cfu/m³. This wide range is partly explained by the impact of outdoor air. Mean levels are, however, typically 102-103 cfu/m³. In two studies, lower indoor levels have been reported in winter; this was noted not only in a cold climate (Reponen et al., 1992) but also in a subtropical climate (Kuo and Li, 1994). In most studies, the sampling period covered several seasons. The impact of the seasonal variation has either not been taken into account in the studies of indoor fungi or has been resolved by calculating indoor/outdoor ratios of total fungi or genera. The total concentrations of 28 fungal spores in homes varied between $10^3 – 6{\cdot}10^5$ spores/m³ (Bjornsson et al., 1995; Rautiala et al., 1996; Toivola et al., 2002). As examples of other environments, fungal levels in a few studies concerning offices and a hotel varied between 10-10^4 cfu/m³.

The most frequently found genus in indoor air has been *Penicillium* together with *Cladosporium*, *Aspergillus* (Hunter et al., 1988; Miller et al., 1988; Strachan et al., 1990; Pasanen, 1992; Kuo and Li, 1994; Gorny, Dutkiewicz and Krysinska-Traczyk, 1999) and yeasts (Hunter et al., 1988; Pasanen et al., 1992b; Pasanen, 1992). These common genera and groups are mostly the same, independent of the climate or continent, because the studies originate from Great Britain, Canada, the Netherlands, Finland, Taiwan, Belgium, Norway, USA, and Poland. In several studies, *Cladosporium* has been the most dominating genus and its main source has been outdoor air (Verhoeff et al., 1992; Dharmage et al., 1999; Su et al., 2001). In addition to the most common genera or groups, also other genera e.g. *Ulocladium*, *Geomyces*, *Sistotrema* and *Wallemia* have been found relatively often (Hunter et al., 1998; Verhoeff et al., 1992).

6.3 Fungal concentrations in relation to building dampness or moisture

In some studies, the association between elevated fungal levels and moisture damage or observed mould growth has been investigated. There are also a number of reports that present studies of indoor air fungi with disease-based design. The observations of concentrations of viable fungi in moisture damaged residences have been contradictory. In general, fungal concentrations have been higher in moisture damaged buildings than in buildings without such problems (Gallup et al., 1987; Verhoeff et al., 1992; Dharmage et al., 1999; Klanova, 2000). Hunter et al. (1988) also showed that there were higher levels of fungi in a room with visible growth than in those rooms where mould was absent (Hunter et al.). On the other hand, there are many studies where no difference in concentrations of viable fungi between mouldy and non-mouldy buildings has been observed (Strachan et al., 1990; Nevalainen et al., 1991; Pasanen, 1992; Pasanen et al., 1992b; Dill and Niggemann, 1996; Garrett et al., 1998) or between homes with severe and mild mould damage (Miller, Haisley and Reinhardt, 2000). Furthermore, fungal growth in the insulated external wall of precast concrete panel buildings has not been found to affect the indoor air levels (Pessi et al., 2002). In some studies reporting fungal levels, the residences have been defined as complaint buildings with no description on moisture related indoor air problem. In these studies, higher concentrations of fungi or I/O-ratio of the fungal concentration have indicated indoor air sources for fungi (Reynolds, Streifel and Mcjilton, 1990; Dekoster and Thorne, 1995). In addition, fungal levels have been observed to increase during the demolition of mouldy structures or constructional work (Hunter et al., 1988; Rautiala et al., 1996), but decrease back to baseline level in a few months after removal of the damaged materials (Rautiala et al., 1996; Ellringer, Boone and Hendrickson, 2000).

In most studies, the classification of residences is based on reported or observed visible mould. There are only a few studies that have investigated levels in buildings with no moisture or mould damage. The range or average of the fungal concentrations in residences with or without mould or moisture damages have not been always reported, which makes the comparison difficult. In general, the distributions of fungal levels in mouldy and non-mouldy buildings overlap. Extremely high levels (e.g. 23 000 cfu/m³) have been reported even in residences with no visible mould in a study that showed the association between mould damage and fungal levels (Hunter et al., 1988). Only in the studies of Klánová (2000) and Johanning et al. (1999) was the difference in ranges of fungal levels fairly clear. Based on these studies, there is no fungal level that always indicates moisture or mould damage, even though several attempts to set such limits have been reported (Rao, Burge and Chang, 1996). In order to use fungal levels in source characterization, the conclusion must be based on the knowledge of what is considered normal in the environment and climate of interest.

Several studies also deal with residences without any known indoor air problem, in order to describe the overall fungal levels of residences. The levels vary between $10 – 10^5$ cfu/m³ and thus overlap with the levels observed in the residences with moisture or mould problem. These studies have examined several factors accounting for the variation in fungal levels,

such as seasonal variation, outdoor air and ventilation (Kuo and Li, 1994; Gorny, Dutkiewicz and Krysinska-Traczyk, 1999; Pasanen et al., 1989).

6.4 Fungal flora in moisture damaged and reference buildings

Although differences in mean fungal levels between moisture damaged and reference buildings have not always been found, differences in microbial composition of air samples have commonly been noted. For example, higher concentrations of Aspergillus, *Cladosporium*, *Penicillium*, nonsporulating fungi (including basidiomycetes) or yeasts have been observed in buildings with moisture damage or with visible mould growth than in reference buildings (Strachan et al., 1990; Pasanen, 1992; Pasanen et al., 1992b; Dekoster and Thorne, 1995; Garrett et al., 1998). In the study of Miller et al., the total concentrations of viable fungi were similar in residences with severe and mild mould damage, but the presence of severe damage could be seen in the higher prevalence of fungal species not present in the outdoor air (Miller, Haisley and Reinhardt, 2000). Occurrence of certain fungi in air has also been associated with dampness or mould growth in buildings. *Aspergillus versicolor* has been observed frequently in the air of damaged buildings (Hodgson et al., 1998; Jarvis and Morey, 2001). *Stachybotrys* has been noted to occur in a moisture damaged building, but not in the control building (Johanning et al., 1996). In addition, several other genera different from outdoor air have been found, but their occurrence has not been reported to indicate moisture damage. In general, the dominant genera in air have usually been reported, but the value of rare findings as indicators of moisture damage has not been emphasized. However, a list of damage-associated fungi and bacteria has been published as a result of an expert meeting (Samson et al., 2004). This is based on empirical observations, but little published data are available about the frequencies or other characteristics of these microbes in building environments. The list of "indicator microbes", or microbes that do not belong to the normal flora but the presence of which may indicate mould growth is as follows: *Trichoderma*, *Exophiala*, *Phialophora*, *Ulocladium*, *Stachybotrys*, *Fusarium*, *Wallemia*, *Aspergillus versicolor*, *Aspergillus fumigatus*, actinobacteria, gram-negative bacteria and yeasts (e.g. *Rhodotorula* and *Sporobolomyces*) (Samson et al., 2004). Furthermore, the occurrence of different microbes especially in the air should be weighted differently, as some microbes, such as *Stachybotrys chartarum*, *Fusarium* and *Chaetomium* are seldom found airborne due to their spore size and spore formation. However, even these fungi can occasionally be present in abundance in air samples, especially when the fungi are growing prominently in a damage site (Hunter et al., 1988; Etzel et al., 1998; Johanning et al., 1996).

6.5 Fungal concentrations and flora in house dust

House dust samples have been suggested to provide a readily available way to obtain an integrated sample over a long period of time to reflect long-term exposure conditions (Flannigan, 1997; Dillon et al., 1999). Concentrations of viable fungi in dust vary from 10 to 10^8 cfu/g (Miller et al., 1988; Verhoeff et al., 1994a; Koch et al., 2000; Ellringer, Boone and Hendrickson, 2000). The most common genera or groups detected are typically *Penicillium*, yeasts, *Aspergillus*, *Cladosporium* and *Alternaria* (Miller et al., 1988; Verhoeff et al., 1994a; Koch et al., 2000). These are the same ubiquitous fungal genera found also in the air. However, the fungal flora in the house dust may also differ from that present in air, e.g. *Mucor*, *Wallemia* and *Fusarium* have been found frequently in dust samples, but rarely in air (Gravesen et al., 1999; Miller et al., 1988; Ren, Jankun and Leaderer, 1999). According to Koch et al. (2000), the fungal levels and flora in house dust are influenced by the outdoor air fungi and thus show a corresponding seasonal variation, but this was not seen in the study of Ren, Jankun and leader (1999).

Even though a dust sample may represent an integrated sample over a long period of time, a single measurement of viable fungi in house dust does not provide reliable information of exposure due to the low reproducibility and differences in fungal genera compared to the air (Miller et al., 1988; Verhoeff et al., 1994a; Ren, Jankun and Leaderer, 1999).

6.6 Microbes in surface samples

Airborne spores and cells may be deposited onto different surfaces in the indoor environment by gravitational settling or carried by wind currents. Thus, the spores found on indoor surfaces that are not regularly cleaned, may reflect the airborne mycoflora in that indoor environment. Swab sampling from the surface will provide a rough estimate of the airborne flora. Concentrations of viable fungi on surfaces with no visible fungal growth or surfaces with no or minor moisture damage vary from being the under detection limit to approximately 2500 cfu/cm² (Ellringer, Boone and Hendrickson, 2000; Lappalainen et al., 2001). The dominating genera have been *Penicillium* (Macher, Huang and Flores, 1991; Ellringer, Boone and Hendrickson, 2000; Lappalainen et al., 2001) together with *Cladosporium*, yeasts (Macher, Huang and Flores, 1991; Lappalainen et al., 2001), *Aureobasidium* and *Alternaria* (Lappalainen et al., 2001). Interestingly, Aspergillus species have not been commonly found on undamaged surfaces. The fungal concentrations on surfaces nearby visible moisture damage have been reported as 3-260 cfu/cm², within the same range as nearby surfaces without damage (Lappalainen et al., 2001). If there is water available on the surface, fungi will germinate and start to grow. In such situations, the numbers of viable fungi in swab sampling are several orders of magnitude higher than normal background, up to 10^6 cfu/cm² (Johanning et al., 1996; Jarvis and Morey, 2001). The fungal genera observed on visibly damaged surfaces have been *Aspergillus versicolor* (Beguin and Nolard, 1994; Lappalainen et al., 2001; Jarvis and Morey, 2001), *Cladosporium, Penicillium, Ulocladium, Acremonium, Stachybotrys chartarum, Aureobasidium, Trichoderma* and *Scopulariopsis* (Beguin and Nolard, 1994; Lappalainen et al., 2001). There are also studies in which *Stachybotrys* has been the dominating genus found on surfaces (Johanning et al., 1996). Even damp surfaces supporting large populations of bacteria and yeasts or fungal growth do not necessarily result in higher microbial levels in the indoor air (Macher, Huang and Flores, 1991; Buttner and Stetzenbach, 1993). *Stachybotrys* occurring on surfaces is often difficult to detect in the air, whereas easily sporulating genera such as *Aspergillus, Penicillium* and *Cladosporium* have commonly been observed in both types of samples (Cooley et al., 1998; Tiffany and Bader, 2000; Lappalainen et al., 2001). Hence, surface samples are often needed to confirm the findings, even though signs of contamination are seen in air mycoflora of air samples (Reynolds, Streifel and Mcjilton, 1990). Bacterial concentrations on surfaces without visible growth have been noted to be under 4200 cfu/cm² (Lappalainen et al., 2001). Gram-positive rods have been found on both dry and damp surfaces (Macher, Huang and Flores, 1991), while gram-negative rods and actinobacteria have been found in mainly damp or damaged areas (Macher, Huang and Flores, 1991; Lappalainen et al., 2001).

6.7 Microbes in building materials

The concentrations of viable fungi in damaged materials vary typically between 45 – 108 cfu/g (Andersson et al., 1997; Etzel et al., 1998; Johanning et al., 1999; Ellringer, Boone and Hendrickson, 2000; Hodgson et al., 1998; Lappalainen et al., 2001; Pessi et al., 2002). While moisture conditions may fluctuate in the microenvironments of a building, microbial growth is also a complex process regulated by the environmental factors. Therefore, moisture levels and microbial concentrations do not necessarily correlate well in building material samples (Pasanen et al., 2000). The most common fungal genera found in material samples taken from damaged areas have been *Penicillium*, *Aspergillus* (Andersson et al., 1997; Etzel et al., 1998; Gravesen et al., 1999; Ellringer, Boone and Hendrickson,

2000; Lappalainen et al., 2001) *Acremonium, Aspergillus versicolor, Cladosporium* (Ellringer, Boone and Hendrickson, 2000; Flappan et al., 1999; Lappalainen et al., 2001), *Stachybotrys* (Andersson et al., 1997; Gravesen et al., 1999; Johanning et al., 1999; Hodgson et al., 1998), *Chaetomium, Ulocladium* (Gravesen et al., 1999). In addition to these, a number of other genera or species are usually found in damaged materials. The dominant fungal genera found in the material samples are mainly similar to those seen in the air. There are, however, some genera such as *Stachybotrys,*that are not usually found in air (Miller, Haisley and Reinhardt, 2000; Tiffany and Bader, 2000). This is supported by the study of Rautiala et al. (1996), in which some infrequently found genera, such as *Absidia, Botrytis, Exophiala, Fusarium, Graphium*, *Mucor* and *Staphylotrichum*, were found in the damaged materials and also in the air during the dismantling of these materials. Building material samples, showing the actual growth at a given site, have been found to be useful in verifying the sources for the contamination, possibly seen in the air samples (Reynolds, Streifel and Mcjilton, 1990).

7 Prevention and remediation

7.1 Preventing mould

The most effective way to manage mould in a building is to eliminate or limit the conditions that foster its establishment and growth. Every organism has strategies for locating a hospitable environment in obtaining water and nutrients, and reproducing. Intervention in one or more of those strategies can improve the resistance of the environment against microbial contamination.

The key to prevention in the design and operation of buildings is to limit water and nutrients. The two basic methods for accomplishing that are keeping moisture-sensitive materials dry and, when wetting is likely or unavoidable, using materials that offer a poor substrate for growth. Specifically, design and maintenance strategies must be implemented to manage;

- Rainwater and groundwater, preventing liquid water entry and accidental humidification of buildings.
- The distribution, use, disposal of drinking, process, and wash water, making equipment and associated utilities easily accessible for maintenance and repair.
- Water vapour and surface temperatures to avoid accidental condensation.
- The wetting and drying of materials in the building and of soil in crawl spaces during construction.

Existing buildings have more limited options for water and moisture control than new construction because the systems that manage drinking, process, and wash water and that control rainwater, groundwater, water vapour, and heat flow have already been selected and installed. Flawed constituents of existing systems must be repaired, replaced, or addressed through routine operations and maintenance. Operations and maintenance procedures that reduce the likelihood of mould growth include cleaning mould-resistant materials that routinely get wet in the course of ordinary operations (floors in entryways, showers, and condensate systems or cooling coils) and quickly drying mould-prone materials that accidentally get wet through plumbing leaks, rainwater intrusion and the like.

7.2 Published guidance for mould remediation

Efforts to remediate microbial contamination involve direct intervention with building occupants, the source of the contaminant (the mould or other microbial agent), or the transport mechanism, (that is, the means by which a contaminant moves within a building environment). For example, moving people during intense remediation activities is an intervention that involves occupants, removing fungal growth and remediating the moisture problem are interventions that involve the source, depressurizing a mouldy crawl space with fan-powered exhaust intervenes in the transport mechanism, and filtration and increased dilution ventilation intervene in contaminant transport by lowering airborne concentration in general.

Indoor mould has historically been treated as a nuisance contaminant. Two decades ago, there was little guidance for responding to fungal contamination in buildings beyond the general instruction to clean it up. That began to change as more became known about the potential hazards of mould exposures and the practice of remediation. Six guidelines concerning this issue are summarized in “Damp Indoor Spaces and health” (Institute of Medicine, 2004). The documents agree that;

- Mould should not be allowed to colonize materials and furnishing in buildings.
- The underlying moisture condition supporting mould growth should be identified and eliminated.

- The best way to remediate problematic mould growth is to remove it from materials that can be effectively cleaned and to discard materials that cannot be cleaned or are physically damaged beyond use. Managing mould growth in place is not considered by any of the documents
- Occupants and workers must be protected from dampness-related contaminants during remediation. All the guidelines agree that some mould situations present a small enough exposure potential that cleanup does not require specific containment or worker protection but that other situations warrant full containment, air-pressure management, and full worker protection. Situations between those extremes need intermediate levels of care. Guidance for selecting appropriate containment and worker protection for different situations lacks clarity within and between documents.
- HVAC systems are special cases. But the documents disagree on how to respond to contamination in HAVC systems.

The documents are divided on the use of disinfectants. Four recommend that disinfectants be used sparingly, in appropriate locations, for specific purposes, and with caution. The original NYCDOH guidance requires the use of biocides, whereas ISIAQ suggests it for hard surfaces. Only two of the documents – those of ISIAQ and ACGIH – discuss the prevention of mould growth in buildings to any substantial degree.

The American Industrial Hygiene Association (AIHA) document differs from the others in several respects. It identifies itself as supplementary to other guidance, and it is the only document that specifically reviews other guidelines, identifying common ground, disagreements, strengths and weaknesses in the evidence, and gaps in knowledge. It also offers recommendations for best practice. The AIHA document focuses on 11 questions;

1. When should microbial growth found in occupied building be remediated?
2. What amount of mould should indicate what degrees of remediation?
3. What remediation methods should be used?
4. Should biocides be used in remediation?
5. Under what circumstances should buildings be evacuated and work areas isolated?
6. How should remediation work areas be isolated?
7. How should water-damaged items be treated?
8. What quality-assurance principles should be followed to ensure that mould remediation is successful?
9. What personal protective equipment is recommended during remediation?
10. Is personal air sampling appropriate to determine worker exposure during mould remediation?
11. What medical evaluation is recommended for remediators?

A minority report in the AIHA document raises concerns about treating all moulds as hazardous substances and the consequent recommendations for decontamination, worker protection, containment, and disposal.

8 Discussion and conclusions

The relationship between specific health effects and the mould spore concentration has not been well defined (Fung and Hughson, 2003). It has been criticized that the methodologies for sampling and analysis are neither standardized nor definitive (Jarvis and Morey, 2001). Available quantitative methods are used in combination with a comprehensive qualitative assessment (Douwes et al., 2003). Jarvis and Morey (2001) have suggested that lack of a standard methodology is a primary cause for the poorly understood relationship between fungal exposures and health outcomes. Therefore, it is important to be able to identify and quantify the mould contamination levels in indoor environments using validated methods for sampling and analysis.

With the lack of a best practice, one of two approaches is typically used to assess mould contamination with respect to fungal spore identification and enumeration: culture-based analysis (the colony forming unit [CFU] count) and the microscopic analysis (the total spore count). The culture-based analysis, which is more common, gives the ability to identify colonies to the species level and a large reference database is available for proper identification of colonies (Pasanen, 2001). Species-level identification is useful in detecting "indicator fungi" that are commonly found in mouldy buildings.

However, several disadvantages of the CFU analysis are also apparent. The incubation period is usually long (over 7 days for some fungal species) (Dillon et al., 1999; Macher, 2001) and CFU analysis can overlook fungal species that are not easily culturable. Furthermore, it might underrepresent those fungal types that grow slowly because they are overtaken by faster growing colonies (Pasanen, 2001; Macher, 2001; ACGIH, 1999). Some fungal species, such as the spores from Stachybotrys chartartum, have been found to lose their culturability soon after they become airborne; however, this does not appear to affect their allergenicity or toxicity (Miller, 1992; Haugland and Heckman, 1998). Dead micro organisms, cell debris and microbial components are not detected, while they too may have toxic and/or allergenic properties. Health effects, especially respiratory allergies, have been shown to be associated with the total spore count rather than with the CFU count (Strachan et al., 1990).

The main advantage of microscopy is that both dead and living micro organisms are quantified, selection effects are limited, Similar to the CFU count, there are some advantages and disadvantages of the total spore count method. Two advantages are that both viable and nonviable spores can be included, and the total count is less time-consuming than the CFU analysis (can be performed within hours of sample collection). Among disadvantages of this enumeration method, there are masking effects, when the background matrix may mask small spores; high data variability when spore density is low; overestimation of large pigmented spores; and impossibility of performing the species-level identification (Pasanen, 2001).

Other methods for fungal analysis include the use of surrogate markers that measure quantitative loads of fungal biomass, such as β-glucan and ergosterol. These indicator methods are useful for providing general information about the total amount of fungi in the environment but are often not specific enough to relate to health outcomes because of their surrogate nature (ACGIH, 1999). Recently, polymerase chain reaction (PCR) and immunochemical methods have become available for fungal analysis (Haugland and Heckman, 1998; Meklin et al., 2004). There is currently, however, very little reference data available with these techniques.

Currently, there are numerous sampling methods available to measure fungal concentrations in the environment. Source sampling, which includes methods such as swab, tape, bulk, and dust, is commonly used to identify indoor fungi. These source sampling methods have been cited by the American Industrial Hygiene Association (AIHA) (Dillon, Heihnson, and Miller, 1996) as "necessary adjuncts" to air sampling, especially under conditions of low air movement, or when air sampling might result in false-negative findings. However, these surface-based methods cannot identify hidden sources of mould (ACGIH, 1999). Swab and tape sampling are common methods of fungal exposure assessment through the source characterization, partially because of ease of collection. They are often used as tools for identification of fungi but do not provide measures of exposure to airborne spores. Bulk samples include pieces of material such as wallboard, carpet, or return air filter, that are collected from the contaminated area to identify and find the relative concentration of mould in the sample (ACGIH, 1999). Surface sampling is preferred over bulk sampling when a less destructive method of sample collection is desired. Fungal spores can also be measured in settled dust sampled from the floor (Verhoeff et al., 1994). This method is usually attempted to evaluate long-term respiratory exposure to fungi, though the stability of micro organisms over time is questionable (Chew et al., 2003; Verhoeff et al., 1992). Flannigan (1997) indicated that dust may not adequately reflect human inhalation exposure, evidenced by his research findings that only a very small amount of reaerosolized dust particles is of respirable size. Furthermore, Chew et al. (2003) found that culturable air and dust samples represent differing types of potential mould exposure and, thus, are not related indicators of exposure to mould. Settled dust can be analyzed by various techniques, such as CFU, PCR, and biochemical methods for β-glucan and ergosterol. However, it is difficult to conduct the microscopic enumeration from dust samples, in part, because fungi in dust are masked by other particles (Meklin et al., 2004; Chew et al., 2003; Chew et al., 2001).

Air sampling is one of the most common methods used to assess fungal levels in indoor environments. Many studies have related human health effects, such as increases in allergic and asthmatic respiratory symptoms, to airborne fungal spore (Strachan et al., 1990; Johanning et al., 1996). As the health effects of fungal exposure are mainly respiratory, air sampling is believed to be adequate to represent the exposure. However, fungal spores have been found to exhibit varying patterns in their release into the air depending on several environmental factors (Jarvis and Morey, 2001; Chew et al., 2003; Johanning et al., 1996). When air samples are chosen for monitoring, several aspects should be considered: Representativeness of sampling, sampler performance, and possibilities to conducy various analyses. It is well-known that the shorter a sampling time is, the larger is the variability between side-by-samplers, and the lower is the representativeness of sampling (Pasanen, 2001). In total spore sampling personal air sampling is possible and sampling time can be varied over a large range.

The relationship between different fungal assessment methods has not been extensively characterized. Very little information is available on the comparison of the data obtained with the microscopic and culture-based enumeration of samples collected by a specific method, as well as the data collected by different sampling methods.

Most of the methods to measure mould constituents are in an experimental phase and have as yet not been routinely applied and/or are not commercially available. Important advantages of these methods include:

a) the stability of most of the measured components, allowing longer sampling times for airborne measurements, and frozen storage of samples prior to analysis;
b) the use of standards in most of these methods;
c) the enhanced possibility to test for reproducibility.

The interpretation of results is highly controversial. Attempts have been made by various researchers and professional groups to set exposure limits for allergenic spores, but

environments, exposure durations, predisposal health conditions and limitations of viable sampling have made this difficult.
The most recognized approach to defining environmental problems as they relate to microbial allergens is the assessment of genus variability, a process than has as many approaches as there are investigators. It is difficult to say whether one approach is better than another.

The most common approach is to assess percent of total microbial allergens. For instance, the outside air contains 85 percent of *Cladosporium*, 10 percent of *Alternaria*, and 5 percent *Penicillium*, and the indoor air contains 90 percent *Penicillium*, 8 percent *Cladosporium*, and 2 percent *Alternaria*. This example, involving unrealistically limited numbers of mould spores in a total count, is a good indicator that *Penicillium* moulds are growing indoors (Hess-Kosa, 2002)

In issues regarding the prevention of moisture problems and the remediation of buildings that have water damage and microbial contamination, the Institute of Medicine (2004) of the national Academics, USA, gives a summarize of findings and recommendations, in which I totally agree;

Findings

- The most effective way to manage a biological agent, such as mould, in a building is to eliminate or limit the conditions that foster its establishment and growth.
- There are several sources of guidance on how to respond to various indoor microbial contamination situations. However, determining when a remediation effort is warranted or successful is necessarily subjective because there are no generally accepted health-based standards for acceptable concentrations of fungal spores, hyphae, or metabolites in the air or surfaces.
- Remediation must identify and eliminate the underlying cause of dampness or water accumulation. If the underlying causes are not addressed, contamination may recur.
- Valuable information can be acquired from architects, builders, occupants, and maintenance staffs regarding health complaints, the use history of the building, moisture events, and locations of problems. Both expert assessment of building's condition and knowledge of its history and current problems are needed to make a thorough evaluation of potential dampness-related exposure and an effective plan for remediation.
- Fungal and other microbial material is present on nearly all indoor surfaces. There is a great deal of uncertainty and variability in samples taken from indoor air and surfaces, and it may be difficult to discern which organisms are part of the natural background and which are the result of problematic contamination. However, the information gained from a careful and complete survey may aid in the evaluation of contamination sources and remediation needs.
- The potential for exposure to microbial contaminants in spaces such as attics, crawl spaces, exterior sheathing, and garages is poorly understood.
- Disturbance of contaminated material during remediation activities can release microbial particles and result in contamination of clean areas and exposure of occupants and remediation workers.
- Containment has been shown to prevent the spread of moulds, bacteria, and related microbial particles to non contaminated parts of a contaminated building. The amount of containment and worker personal protection and the determination of whether occupant evacuation is appropriate depend on the magnitude of contamination.
- Very few controlled studies have been conducted on the effectiveness of remediation actions in eliminating problematic microbial contamination in the shorter and longer term and on the effect of remediation actions on the health of building occupants.

- Available literature addresses management of microbial contamination when remediation is technically and economically feasible. There are no literature addressing situations where intervening in the moisture dynamic or cleaning or removing contaminated materials is not practicable.

Recommendations

- Homes and other buildings should be designed, operated, and maintained to prevent water intrusion and excessive moisture accumulation when possible. When water intrusion or moisture accumulation is discovered, the source should be identified and eliminated as soon as practicable to reduce the possibility of problematic microbial growth and building material degradation.
- When microbial contamination is found, it should be eliminated by means that limit the possibility of recurrence and limit exposure of occupants and persons conducting the remediation.

9 Further work

9.1 Principal objectives PhD project

This literature review is part of a PhD project concerning moisture in buildings and the risk of mould growth. The principal objectives of the PhD study are to:

- Increase the knowledge and focus on issues related to moisture and mould growth in structures and materials.
- Develop acceptance criteria for moisture safety levels in critical structures and materials.
- Develop guidelines and methods for detection and assessment of mould in buildings in relation to indoor environment

9.2 Activities and methodology

High moisture levels may lead to microbiological growth in structures and materials, and chemical degradation. In either case, the result may be adverse impact on the indoor climate and reduced technical performance. The vulnerability of selected building materials and different constructions to microbiological growth will be emphasised in particular. Through systematic investigations, field surveys and laboratory experiments we aim in the PhD project to further develop the knowledge on this issue. The PhD study is divided into the following main activities:

A. State-of-the-art analysis on measurement strategy and analytical methods used to describe microbiological growth. This analysis is based on a literary study. Today there are no Norwegian or international standards on how to handle moisture damage and microbiological growth. This study might give a contribution to this work.

B. State-of-the-art analysis on the relationship between moisture in structures and materials and microbiological growth. This analysis will include a literary study, a field study and an experimental study. In the field study we will explore roofs where moist have been build inn during construction. In the experimental study we compare the growth in roof-elements where we have added an amount of water and spores from mould, to see the effect of varying moist and temperature. The elements are actually roof-elements on our research facility house at Voll. Some of the elements are modified in a way that they have airing facility. The humidity and temperature in these elements are influenced of the outside weather conditions. As a control we are going to build two roof-elements (one with airing facility and one without) inside our lab. These elements are being handled the same way as the one outside. But these elements have constant temperature and humidity in the surrounding air.

   Temperature and humidity are the limiting factors (except for nutrition) for mould growth. By exposing different materials to spores and varying temperature and humidity we can get a growth-curve for the specific material to the specific mould species.

C. Investigation on how physical factors in the building influence indoor air quality on spores and microbiological volatile organic compounds (MVOC) from mould. Through field surveys record and analyse these factors compared to the registered moisture, mould and MVOC conditions in the building. We also would like to investigate how hidden mould growth in an outer wall can influence on the spore and MVOC concentration in the indoor air. By building a wall in our "emission chamber" (a chamber where the air is filtered through a HEPA filter, and the air is almost completely free of particles) we can do controlled experiments on which factors (e.g. pressure over the wall) that influence possible spore and MVOC transport through out the wall.

9.3 Utilitarian value and expected results

The PhD study will lead to an increase in the knowledge about how moist influences microbiological growth on different materials and structures. This is important for the indoor climate, and will lead to a higher level of reliability in buildings, extended lifetime, reduced administration, damage and maintenance costs through correct planning and building design.

The study will be an important contribution to the pre-normative research for the continued development of Norwegian and international standards.

References

ACGIH. *Bioaerosols: Asessment and Control*. Cinncinati, OH: ACGIH, 1999.

Aketagawa, J., et al. "Activation of Limulus Coagulation Factor-G by Several (1-3)-Beta-D-Glucans - Comparison of the Potency of Glucans with Identical Degree of Polymerization But Different Conformations." *Journal of Biochemistry* 113.6 (1993): 683-86.

Alvarez, A. J., et al. "Use of Solid-Phase Pcr for Enhanced Detection of Airborne Microorganisms." *Applied and Environmental Microbiology* 60.1 (1994): 374-76.

Andersson, M. A., et al. "Bacteria, molds, and toxins in water-damaged building materials." *Applied and Environmental Microbiology* 63.2 (1997): 387-93.

Arbes, S. J., et al. "Abatement of cockroach allergen (Bla g 1) in low-income, urban housing: A randomized controlled trial." *Journal of Allergy and Clinical Immunology* 112.2 (2003): 339-45.

Barnes, C., et al. "Allergenic materials in the house dust of allergy clinic patients." *Annals of Allergy Asthma & Immunology* 86.5 (2001): 517-23.

Beguin, H and N Nolard. "Mould biodiversity in homes I. Air and surface analysis of 130 dwellings." *Aerobiology* 11 (1994): 65-68.

Biagini, R. E. *Bioaerosols, Fungi and Mycotoxins: Health Effects, Assessment, Prevention and Control*. Ed. Johanning E. Albany: Eastern New York Occupational and Environmental health Centre, 1999, 320-29.

Bjornsson, E., et al. "Asthmatic Symptoms and Indoor Levels of Microorganisms and House-Dust Mites." *Clinical and Experimental Allergy* 25.5 (1995): 423-31.

Bünger, J, et al. *Bioaerosols, Fungi and Mycotoxins: Health Effects, Assessment, Prevention and Control*. Ed. Johanning E. Albany: Eastern New York Occupational and Environmental health Centre, 1999, 112-18.

Buttner, M. P. and L. D. Stetzenbach. "Monitoring Airborne Fungal Spores in An Experimental Indoor Environment to Evaluate Sampling Methods and the Effects of Human Activity on Air Sampling (Vol 59, Pg 226, 1993)." *Applied and Environmental Microbiology* 59.5 (1993): 1694.

Cage, B. R., et al. "Evaluation of four bioaerosol samplers in the outdoor environment." *Annals of Allergy Asthma & Immunology* 77.5 (1996): 401-06.

Chang, J. C. S, K. K. Foarde and D. W. Vanosdell. "Growth evaluation of fungi (penicilium and aspergillus spp.) on ceiling tiles." *Atmospheric Environment* 29.17 (1995): 2331-37.

Chew, G. L., et al. "Fungal extracellular polysaccharides, beta (1 -> 3)-glucans and culturable fungi in repeated sampling of house dust." *Indoor Air* 11.3 (2001): 171-78.

Chew, G. L., et al. "Dustborne and airborne fungal propagules represent a different spectrum of fungi with differing relations to home characteristics." *Allergy* 58.1 (2003): 13-20.

Cooley, J. D., et al. "Correlation between the prevalence of certain fungi and sick building syndrome." *Occupational and Environmental Medicine* 55.9 (1998): 579-84.

Croft, W. A., et al. "Clinical confirmation of trichothecene mycotoxicosis in patient urine." *Journal of Environmental Biology* 23.3 (2002): 301-20.

Crook, B. "Review: Methods of monitoring for process micro-organisms in biotechnology." *Annals of Occupational Hygiene* 40.3 (1996): 245-60.

Dekoster, J. A. and P. S. Thorne. "Bioaerosol Concentrations in Noncomplaint, Complaint, and Intervention Homes in the Midwest." *American Industrial Hygiene Association Journal* 56.6 (1995): 573-80.

Dharmage, S., et al. "Prevalence and residential determinants of fungi within homes in Melbourne, Australia." *Clinical and Experimental Allergy* 29.11 (1999): 1481-89.

Dill, I. and B. Niggemann. "Domestic fungal viable propagules and sensitization in children with IgE mediated allergic diseases." *Pediatric Allergy Immunology*. 7.3 (1996): 151-55.

Dillon, H. K., P. A. Heihnson and J. D Miller. "Viable Fungi and Bacteria in Air, Bulk, and Surface Samples." *Guide for the Determination of Biological Contaminants in Environmental Samples*. American Industrial Hygiene Association (AIHA), 1996. 31-44.

Dillon, H. K., et al. "Review of methods applicable to the assessment of mold exposure to children." *Environmental Health Perspectives* 107 Suppl 3 (1999): 473-80.

Douwes, J., et al. "Measurement of beta(1->3)-glucans in occupational and home environments with an inhibition enzyme immunoassay." *Applied and Environmental Microbiology* 62.9 (1996): 3176-82.

Douwes, J., et al. "Bioaerosol health effects and exposure assessment: Progress and prospects." *Annals of Occupational Hygiene* 47.3 (2003): 187-200.

Douwes, J., et al. "Fungal extracellular polysaccharides in house dust as a marker for exposure to fungi: relations with culturable fungi, reported home dampness, and respiratory symptoms." *Journal of Allergy and Clinical Immunology* 103.3 Pt 1 (1999): 494-500.

Duchaine, G, Grimard, Y and Cormier, Y. Influence of building maintenance factors and seasons on airborne contaminants of swine confinement buildings. *American Industrial Hygiene Association Journal* 61, 361-66. 2000.

Eduard, W. and O. Aalen. "The Effect of Aggregation on the Counting Precision of Mold Spores on Filters." *Annals of Occupational Hygiene* 32.4 (1988): 471-79.

Eduard, W. and D. Heederik. "Methods for quantitative assessment of airborne levels of noninfectious microorganisms in highly contaminated work environments." *American Industrial Hygiene Association Journal* 59.2 (1998): 113-27.

Elke, K., et al. "Determination of selected microbial volatile organic compounds by diffusive sampling and dual-column capillary GC-FID--a new feasible approach for the detection of an exposure to indoor mould fungi?" *Journal of Environmental Monitoring* 1.5 (1999): 445-52.

Ellringer, P. J., K. Boone and S. Hendrickson. "Building materials used in construction can affect indoor fungal levels greatly." *American Industrial Hygiene Association Journal* 61.6 (2000): 895-99.

Etzel, R. A., et al. "Acute pulmonary hemorrhage in infants associated with exposure to Stachybotrys atra and other fungi." *Archives of Pediatric and Adolescent Medicine*. 152.8 (1998): 757-62.

Eudey, L, H. J. Su and H. A. Burge. "Biostatistics and Bioaerosols." *Bioaerosols*. Ed. H. A. Burge. Lewis Publishers, 1995. 269-307.

Fischer, G. and W. Dott. "Relevance of airborne fungi and their secondary metabolites for environmental, occupational and indoor hygiene." *Archives of Microbiology* 179.2 (2003): 75-82.

Fischer, G., et al. "Species-specific production of microbial volatile organic compounds (MVOC) by airborne fungi from a compost facility." *Chemosphere* 39.5 (1999): 795-810.

Flannigan, B. "Air sampling for fungi in indoor environments." *Journal of Aerosol Science* 28.3 (1997): 381-92.

Flappan, S. M., et al. "Infant pulmonary hemorrhage in a suburban home with water damage and mold (Stachybotrys atra)." *Environmental Health Perspectives* 107.11 (1999): 927-30.

Fung, F. and W. G. Hughson. "Health effects of indoor fungal bioaerosol exposure." *Applied Occupational and Environmental Hygiene*. 18.7 (2003): 535-44.

Gallup, J., et al. "Indoor mold spore exposure: characteristics of 127 homes in southern California with endogenous mold problems." *Experientia Supplementum* 51 (1987): 139-42.

Garrett, M. H., et al. "Indoor airborne fungal spores, house dampness and associations with environmental factors and respiratory health in children." *Clinical and Experimental Allergy* 28.4 (1998): 459-67.

Gorny, R. L., J. Dutkiewicz and E. Krysinska-Traczyk. "Size distribution of bacterial and fungal bioaerosols in indoor air." *Annals of Agricultural and Environmental Medicine*. 6.2 (1999): 105-13.

Grant, C., et al. "The Moisture Requirements of Molds Isolated from Domestic Dwellings." *International Biodeterioration* 25.4 (1989): 259-84.

Gravesen, S., et al. "Microfungal contamination of damp buildings--examples of risk constructions and risk materials." *Environmental Health Perspectives* 107 Suppl 3 (1999): 505-08.

Harrach, B., et al. "Isolation of Satratoxins from the Bedding Straw of A Sheep Flock with Fatal Stachybotryotoxicosis." *Applied and Environmental Microbiology* 45.5 (1983): 1419-22.

Haugland, R. A. and J. L. Heckman. "Identification of putative sequence specific PCR primers for detection of the toxigenic fungal species Stachybotrys chartarum." *Molecular and Cellular Probes* 12.6 (1998): 387-96.

Hess-Kosa, K. *Indoor Air Quality - Sampling Methodologies*. Lewis Publishers, 2002.

Hodgson, M. J., et al. "Building-associated pulmonary disease from exposure to Stachybotrys chartarum and Aspergillus versicolor." *Journal of Occupational and Environmental Medicine* 40.3 (1998): 241-49.

Hunter, C. A., et al. "Mold in Buildings - the Air Spora of Domestic Dwellings." *International Biodeterioration* 24.2 (1988): 81-101.

Hyvärinen, A. *Characterizing Moisture Damaged Buildings - Environmental and Biological Monitoring*. Diss. University of Kuopio, 2002.

Institute of Medicine. *Damp Indoor Spaces and Health*. Institute of Medicine of the National Academies, 2004.

Jarvis, B. B. "Stachybotrys chartarum: a fungus for our time." *Phytochemistry* 64.1 (2003): 53-60.

Jarvis, B. B. and J. D. Miller. "Mycotoxins as harmful indoor air contaminants." *Applied Microbiology and Biotechnology* 66.4 (2005): 367-72.

Jarvis, J. Q. and P. R. Morey. "Allergic respiratory disease and fungal remediation in a building in a subtropical climate." *Applied Occupational and Environmental Hygiene*. 16.3 (2001): 380-88.

Jensen, P. A. "Evaluation of Standard and Modified Sampling Heads for the International Pbi Surface Air System Bioaerosol Samplers." *American Industrial Hygiene Association Journal* 56.3 (1995): 272-79.

Johanning, E., et al. "Health and immunology study following exposure to toxigenic fungi (Stachybotrys chartarum) in a water-damaged office environment." *International Archives of Occupational and Environmental Health* 68.4 (1996): 207-18.

Johanning, E., et al. "Clinical experience and results of a sentinel health investigation related to indoor fungal exposure." *Environmental Health Perspectives* 107 (1999): 489-94.

Karlsson, K. and P. Malmberg. "Characterization of Exposure to Molds and Actinomycetes in Agricultural Dusts by Scanning Electron-Microscopy, Fluorescence Microscopy and the Culture Method." *Scandinavian Journal of Work Environment & Health* 15.5 (1989): 353-59.

Kemp, P. C., et al. "Changes in airborne fungi from the outdoors to indoor air; Large HVAC systems in nonproblem buildings in two different climates." *American Industrial Hygiene Association Journal* 64.2 (2003): 269-75.

Khan, A. A. and C. E. Cerniglia. "Detection of Pseudomonas-Aeruginosa from Clinical and Environmental-Samples by Amplification of the Exotoxin-A Gene Using Pcr." *Applied and Environmental Microbiology* 60.10 (1994): 3739-45.

Klànovà, K. "The concentrations of mixed populations of fungi in indoor air: rooms with and without mould problems; rooms with and without health complaints." *Central European Journal of Public Health* 8.1 (2000): 59-61.

Koch, A., et al. "Indoor viable mold spores--a comparison between two cities, Erfurt (eastern Germany) and Hamburg (western Germany)." *Allergy* 55.2 (2000): 176-80.

Kuo, Y. M. and C. S. Li. "Seasonal Fungus Prevalence Inside and Outside of Domestic Environments in the Subtropical Climate." *Atmospheric Environment* 28.19 (1994): 3125-30.

Lagana, A., et al. "Liquid chromatography/tandem mass spectrometry for the identification and determination of trichothecenes in maize." *Rapid Communications in Mass Spectrometry* 17.10 (2003): 1037-43.

Lappalainen, S, O Lindroos and K Reijula. "Concentrations of Airborne Viable Spores of Fungi and Actinomycetes in Mold Damaged Offices and Schools." *Bioaerosols, Fungi and Mycotoxins; Health Effects, Assessment, Prevention and Control*. Ed. Johanning E. 1999. 616-19.

Lappalainen, S., et al. "Evaluation of priorities for repairing in moisture-damaged school buildings in Finland." *Building and Environment* 36.8 (2001): 981-86.

Ludwig, J. A and J. F. Reynolds. "Diversity Indices." *Statistical Ecology*. New York: John Wiley & Sons, 1988. 85-103.

Ludwig, J. A. and J. F. Reynolds. "Interspecies Association." *Statistical Ecology*. New York: John Wiley & Sons, 1988. 85-103.

Macher, J. "Data Analysis". *Bioaerosols Assessment and Control Cincinnati*, Ohio: American Conference of Govermental Industrial Hygienists, 1999.V 13-1-13-16.

Macher, J. M. "Review of Methods to Collect Settled Dust and Isolate Culturable Microorganisms." *Indoor Air* 11.2 (2001): 99-110.

Macher, J. M., F. Y. Huang and M. Flores. "A 2-Year Study of Microbiological Indoor Air-Quality in A New Apartment." *Archives of Environmental Health* 46.1 (1991): 25-29.

Magurran, A. E. "Diversity Indices and Species Abundance Models." *Ecological Diversity and Its Measurements*. Princeton University Press, 1988. 8-45.

Meklin, T., et al. "Quantitative PCR analysis of house dust can reveal abnormal mold conditions." *Journal of Environmental Monitoring* 6.7 (2004): 615-20.

Meklin, T., et al. "Effect of building frame and moisture damage on microbiological indoor air quality in school buildings." *American Industrial Hygiene Association Journal* 64.1 (2003): 108-16.

Miller, J. D, P Dales and J White. *Bioaerosols, Fungi and Mycotoxins: Health Effects, Assessment, Prevention and Control*. Ed. Johanning E. Albany: Eastern New York Occupational and Environmental health Centre, 1999, 298-305.

Miller, J. D. "Fungi As Contaminants in Indoor Air." *Atmospheric Environment Part A-General Topics* 26.12 (1992): 2163-72.

Miller, J. D., P. D. Haisley and J. H. Reinhardt. "Air sampling results in relation to extent of fungal colonization of building materials in some water-damaged buildings." Indoor *Air-International Journal of Indoor Air Quality and Climate* 10.3 (2000): 146-51.

Miller, J. D., et al. "Fungi and Fungal Products in Some Canadian Houses." *International Biodeterioration* 24.2 (1988): 103-20.

Miller, J. D. and J. C. Young. "The use of ergosterol to measure exposure to fungal propagules in indoor air." *American Industrial Hygiene Association Journal* 58.1 (1997): 39-43.

Murtoniemi, T., et al. "Induction of cytotoxicity and production of inflammatory mediators in raw264.7 macrophages by spores grown on six different plasterboards." *Inhal.Toxicol*. 13.3 (2001): 233-47.

Nevalainen, A., et al. "The Indoor Air-Quality in Finnish Homes with Mold Problems." *Environment International* 17.4 (1991): 299-302.

Nevalainen, A., et al. "Performance of Bioaerosol Samplers - Collection Characteristics and Sampler Design Considerations." *Atmospheric Environment Part A-General Topics* 26.4 (1992): 531-40.

Pasanen, A. L. "Airborne Mesophilic Fungal Spores in Various Residential Environments." *Atmospheric Environment Part A-General Topics* 26.16 (1992): 2861-68.

Pasanen, A. L., et al. "Fungal Microcolonies on Indoor Surfaces - An Explanation for the Base-Level Fungal Spore Counts in Indoor Air." *Atmospheric Environment Part B-Urban Atmosphere* 26.1 (1992): 117-20.

Pasanen, A. L., et al. "Fungi Carried from Farmers Work Into Farm Homes." *American Industrial Hygiene Association Journal* 50.12 (1989): 631-33.

Pasanen, A. L., et al. "Airborne Cladosporium and Other Fungi in Damp Versus Reference Residences." *Atmospheric Environment Part B-Urban Atmosphere* 26.1 (1992): 121-24.

Pasanen, A. L., et al. "The relationship between measured moisture conditions and fungal concentrations in water-damaged building materials." *Indoor Air* 10.2 (2000): 111-20.

Pasanen, A. L. "A Review: Fungal Exposure Assessment in Indoor environments." *Indoor Air* 11 (2001): 87-98.

Pessi, A. M., et al. "Microbial growth inside insulated external walls as an indoor air biocontamination source." *Applied and Environmental Microbiology* 68.2 (2002): 963-67.

Portnoy, J. M., C. S. Barnes and K. Kennedy. "Sampling for indoor fungi." *Journal of Allergy and Clinical Immunology* 113.2 (2004): 189-98.

Rao, C. Y., H. A. Burge and J. C. S. Chang. "Review of quantitative standards and guidelines for fungi in indoor air." *Journal of the Air & Waste Management Association* 46.9 (1996): 899-908.

Rautiala, S., et al. "Exposure to airborne microbes during the repair of moldy buildings." *American Industrial Hygiene Association Journal* 57.3 (1996): 279-84.

Rautiala, S. H., A. I. Nevalainen and P. J. Kalliokoski. "Firefighting efforts may lead to massive fungal growth and exposure within one week. A case report." *International Journal of Occupational Medicine and Environmental Health* 15.3 (2002): 303-08.

Ren, P., T. M. Jankun and B. P. Leaderer. "Comparisons of seasonal fungal prevalence in indoor and outdoor air and in house dusts of dwellings in one Northeast American county." *Journal of Exposure Analysis and Environmental Epidemiology*. 9.6 (1999): 560-68.

Reponen, T., et al. "Normal Range Criteria for Indoor Air Bacteria and Fungal Spores in a Subarctic Climate." *Indoor Air* 2.1 (1992): 26-31.

Reynolds, S. J., A. J. Streifel and C. E. Mcjilton. "Elevated Airborne Concentrations of Fungi in Residential and Office Environments." *American Industrial Hygiene Association Journal* 51.11 (1990): 601-04.

Rogers, C. A. "Indoor fungal exposure." *Immunology and Allergy Clinics of North America* 23.3 (2003): 501-518.

Ruiz-Herrera, J. "Biosynthesis of beta-glucans in fungi." *Antonie Van Leeuwenhoek* 60.2 (1991): 72-81.

Rylander, R. *Bioaerosols, Fungi and Mycotoxins: Health Effects, Assessment, Prevention and Control*. Ed. Johanning E. Albany: Eastern New York Occupational and Environmental health Centre, 1999, 28-32.

Rylander, R. and P. G. Holt. "(1 -> 3)-beta-D-glucan and endotoxin modulate immune response to inhaled allergen." *Mediators of Inflammation* 7.2 (1998): 105-10.

Rylander, R. and R. H. Lin. "(1 -> 3)-beta-D-glucan - relationship to indoor air-related symptoms, allergy and asthma." *Toxicology* 152.1-3 (2000): 47-52.

Samson, R. et al. "Recommendations, in: Health implications of fungi in indoor environments." *Elsevier Science, Amsterdam* (2004): 529-538.

Schnurer, J. "Comparison of Methods for Estimating the Biomass of 3 Food-Borne Fungi with Different Growth-Patterns." *Applied and Environmental Microbiology* 59.2 (1993): 552-55.

Shelton, B. G., et al. "Profiles of airborne fungi in buildings and outdoor environments in the United States." *Applied and Environmental Microbiology* 68.4 (2002): 1743-53.

Spicer, R. C. and H. J. Gangloff. "Limitations in application of Spearman's rank correlation to bioaerosol sampling data." *American Industrial Hygiene Association Journal* 61.3 (2000): 362-66.

Strachan, D. P., et al. "Quantification of Airborne Molds in the Homes of Children with and Without Wheeze." *Thorax* 45.5 (1990): 382-87.

Su, H. J., et al. "Exposure assessment of indoor allergens, endotoxin, and airborne fungi for homes in southern Taiwan." *Environmental Research* 85.2 (2001): 135-44.

Thorne, P. S., et al. "Bioaerosol Sampling In-Field Studies - Can Samples be Express Mailed." *American Industrial Hygiene Association Journal* 55.11 (1994): 1072-79.

Tiffany, J. A. and H. A. Bader. "Detection of Stachybotrys chartarum: The effectiveness of culturable-air sampling and other methods." *Journal of Environmental Health* 62.9 (2000): 9-11.

Toivola, M., et al. "Personal exposures and microenvironmental concentrations of particles and bioaerosols." *Journal of Environmental Monitoring* 4.1 (2002): 166-74.

Verhoeff, A. P. and H. A. Burge. "Health risk assessment of fungi in home environments." *Annals of Allergy Asthma & Immunology* 78.6 (1997): 544-54.

Verhoeff, A. P., et al. "Fungal Propagules In-House Dust .1. Comparison of Analytic Methods and Their Value As Estimators of Potential Exposure." *Allergy* 49.7 (1994): 533-39.

Verhoeff, A. P., et al. "Enumeration and Identification of Airborne Viable Mold Propagules in Houses - A Field Comparison of Selected Techniques." *Allergy* 45.4 (1990): 275-84.

Verhoeff, A. P., et al. "Presence of Viable Mold Propagules in Indoor Air in Relation to House Damp and Outdoor Air." *Allergy* 47.2 (1992): 83-91.

Verhoeff, A. P., et al. "Fungal Propagules In-House Dust .2. Relation with Residential Characteristics and Respiratory Symptoms." *Allergy* 49.7 (1994): 540-47.

Viitanen, H. A. "Modelling the time factor in the development of mould fungi - The effect of critical humidity and temperature conditions on pine and spruce sapwood." *Holzforschung* 51.1 (1997): 6-14.

Walinder, R., et al. "Nasal lavage biomarkers: Effects of water damage and microbial growth in an office building." *Archives of Environmental Health* 56.1 (2001): 30-36.

Zwick, H., et al. "Personal Spore Sampling and Indirect Immunofluorescent Test for Exploration of Hypersensitivity Pneumonitis Due to Mold Spores." *Allergy* 46.4 (1991): 277-83.