OF MICROBES AND MEN

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ABSTRACT

The moisture accumulation, microbial ecology of the damage site and exposure to biological pollutants are all complex phenomena, and may result in various cases of indoor air pollution and health outcomes. Commonly reported are irritation symptoms, respiratory infections and general symptoms, but also diseases have been linked to mold, e.g. increased risk of asthma and clusters of hypersensitivity pneumonitis, pulmonary hemorrhage of infants and rheumatic diseases. Causal agents, still poorly known, may be linked to the complex interactions with the growth substrate and other microbes. Fungi and bacteria growing on building materials produce toxic secondary metabolites, and the material seems to have a critical effect on their production. Modern building technology has provided new ecological niches for microbes which readily exploit the faults in moisture control. In order to better describe the microbial exposures in buildings, current method development focuses on chemical markers of the biomass and PCR.

INDEX TERMS

Fungi, bacteria, health effects, microbial growth, exposure assessment

INTRODUCTION

"All happy families resemble one another, but each unhappy family is unhappy in its own way" (Tolstoy, 1877)

Microorganisms are present everywhere, being part of our normal environment. They are present in soil, air, waters, animals and plants. Human body has more bacterial cells than human cells. Microbes are also present in indoor environments; in air, in materials and on all surfaces, unless continuously kept sterile. The environmental conditions including nutrients, moisture, temperature and other factors regulate the growth in each microenvironment. Microbes and humans are both occupants of indoor environments, and in a healthy building, they live side by side without causing harm to each other. Microbes have their normal sources, such as outdoor air, humans and their activities (Lighthart and Mohr, 1994), but they are also continuously removed from the indoor air by ventilation, gravitational settling and other such mechanisms. In normal conditions, they do not notably grow in the materials, structures or dry surfaces of the indoor environment.

Since we humans build the houses for our own use and benefit, the conditions are meant to be ideal for us. In case this fails and the conditions are more favorable to microbes than man, there is an immediate conflict of interest. This happens if the building provides moisture for the microbes to grow. The development of a moisture damage is a complex phenomenon, as are the microbial ecological phenomena of the damage site, the characteristics of the exposure and the resulting health outcomes. Therefore, each moisture and mold damaged building is different from another case and may lead to different outcomes.

HEALTH EFFECTS

The association between dampness or moisture damage of buildings, mold, microbial growth and adverse health effects has been shown in over 100 reports documenting the phenomenon, as reviewed by e.g., Peat *et al.* (1998) and Bornehag *et al.* (2001). The health outcomes in buildings with different moisture and mold damage vary greatly. Most of the adverse health effects associated with moldy buildings are irritation symptoms, recurrent respiratory infections and unspecific, neurological or general symptoms (Dales *et al.*, 1991).

However, also diseases are connected with indoor mold exposure. An increased risk of asthma has been shown; the risk for asthma symptoms being 1.5-3.5 (Peat *et al.*, 1998, Dekker *et al.*, 1991, Maier *et al.*, 1997, Lindfors *et al.*, 1995, Williamson *et al.*, 1997). Cases or clusters of hypersensitivity pneumonitis (Apostolakos *et al.*, 2001, Trout *et al.*, 2001), pulmonary hemorrhage of infants (Montana *et al.*, 1995, Flappan *et al.*, 1999) and even rheumatic diseases (Seuri *et al.*, 1998) have been associated with indoor exposure to dampness, moisture or mold.

Although cases of allergic asthma due to mold exposure in occupational settings have been described (Fung *et al.*, 2000), allergy to mold due to indoor exposure is relatively rare, the prevalence being approximately 5% (Taskinen *et al.*, 1997, Immonen *et al.*, 2001). Therefore, many of the outcomes may have other pathophysiological mechanisms, still poorly known. Preliminary observations speak for unspecific inflammation (Hirvonen *et al.*, 1999, Walinder *et al.*, 2001, Purokivi *et al.*, 2001, Roponen *et al.*, 2002). There is probably no single causal agent behind the observed health effects but rather a variety of agents consisting of irritating, allergenic, toxic, inflammatory and immunologically active substances.

It is interesting that similar health outcomes have been reported in different climates and cultures, although the primary causes of moisture damage must vary due to different climatic conditions. This may be linked to the fact that modern construction techniques and building materials, such as concrete, plastics, glues, chipboards, plasterboards, wallpapers and insulation materials are commonly used everywhere. Once such a material is moistened to allow microbial growth, the conditions provided are specific for the microenvironment in question, but they are not necessarily specific to any geographical or climatic region. Thus the microbial ecological phenomena in moisture damaged building materials may be universal by their nature.

CAUSES OF DAMPNESS AND MOISTURE DAMAGE

The dynamics, causes and consequences of dampness or moisture problems of buildings are complex and diverse. In general, water may transfer and accumulate into building materials by liquid flow from leaks, condensation, capillary movement, air movement or vapor diffusion (Oliver, 1997). It may involve an acute event, as in cases of plumbing leaks or flooding, or as the other extreme, gradual and fluctuating accumulation of outdoor air humidity due to condensation. In cases of inadequate ventilation, condensation of indoor humidity takes place. Different mechanisms of water intrusion result in different moisture content of materials and thus provide varying conditions for microbial growth. The moist material may also dry and become wet again, thus providing fluctuating moisture conditions (Pasanen *et al.*, 2000). Dampness and moisture in buildings are complex phenomena, and different ways to define the moisture status of the buildings can be used (Chelelgo *et al.*, 2001). Little is so far understood about the role of individual moisture characteristics in the development of adverse exposures and health effects, but the extent of moisture damage has a dose-responsive relationship with the occupants' symptoms, i.e., the more extensive damage, the more symptoms (Haverinen *et al.*, 2001).

THE VARIETY OF SOURCES – MICROBIOLOGY OF BUILDING MATERIALS

Once a building material is moistened, microbes may readily grow on it. While the availability of moisture is the primary factor regulating the growth, the characteristics of the substrate and prevailing environmental conditions affect the dynamics of the growth. The building material itself may act as the substrate for the growth. A study characterizing different building materials, visibly damaged by moisture and microbial growth, showed remarkable differences in their fungal profiles (Hyvärinen et al., 2002). Wooden samples had the greatest diversity of fungi, while plasterboard and ceramic materials showed notably smaller number of fungal genera. Stachybotrys was significantly associated with plasterboard, and Acremonium with ceramic materials. However, in no case was the growth a pure culture of a single fungus; the fungal diversity was remarkable in all the materials, and in most cases of fungal growth, also bacteria were present. The great diversity of microbial genera and species is a general and natural phenomenon in most environments, and is shown to be true also in infested building materials. Therefore, the characteristics of microbial growth in each case of damage may vary substantially, and probably no two damage sites are identical for their microbiological nature.

The emissions from the material with microbial growth also vary. The release of fungal spores from e.g. ceiling tile surfaces is dependent on the air humidity and velocity above the surface, texture of the surface, vibration of the contaminated material and on the fungal species in question (Foarde *et al.*, 1999, Górny *et al.*, 2001). Therefore, the emission of the spores from a site of growth is no even and continuous process, but affected by a number of factors. Thus, the source strength of a moldy damage site may vary remarkably depending on the combination of conditions in each case.

PRODUCTION OF SECONDARY METABOLITES

Microbes are not only harmoniously growing together in a moistened material, but they have complex interactions with both the substrate and with their neighboring microbes. Many fungi and bacteria detected in damaged building materials have remarkable metabolic capacity to produce both volatile end products of their metabolism (MVOC)

(Sunesson *et al.*, 1996, Korpi *et al.*, 1999), and more complex substances called as secondary metabolites. Many of these substances are exploited by humans as antibiotics and other pharmaceutical products, but among the microbial secondary metabolites are also many acutely toxic compounds. Microbial toxins are produced both by fungi (Gravesen *et al.*, 1998) and by bacteria (Andersson *et al.*, 1998, Peltola *et al.*, 2001). Toxins are regularly detected in mold-infested building materials (Tuomi *et al.*, 2001, Nielsen *et al.*, 2001) and thus, their production seems to be a common phenomenon in buildings. However, not all strains of toxigenic fungi or bacteria produce toxins; e.g., only part of the strains of *Stachybotrys chartarum* isolated from indoor environments are toxin producers (Ruotsalainen *et al.*, 1998, Jarvis *et al.*, 1998).

The substrate, or the material the organism is growing, may have a critical effect on the production of toxins and other substances with biological activity. A *Streptomyces sp.* strain was grown in experimental conditions on wood, chipboard, plasterboard, concrete and mineral wool (Roponen *et al.*, 2001). The produced spores were collected and their toxicity and inflammatory potential measured in an *in vitro* setting using mouse macrophages. Both the cytotoxicity and inflammatory potential of the spores varied according to the material on which the spores had been produced. Similar variation in toxicity and other biological potential has been shown in experiments with plasterboard and strains of *Penicillium sp, Aspergillus versicolor, Stachybotrys chartarum* and *Streptomyces sp.* (Murtoniemi *et al.*, 2001). The presence of various toxins in damaged building materials have been shown e.g., by Nielsen *et al.*(1999) and by Tuomi *et al.* (2000).

Toxins may be carried into the indoor air by the spores of the toxigenic fungi (Croft *et al.*, 1986, Smith *et al.*, 1992), but few reports have presented results from measurements of toxins directly from air. At present, the exposure characteristics are not sufficiently known to allow conclusions about a causal connection between the toxic substances originating from microbial sources and health effects of building occupants. However, the present results suggest that the material of the damage site may have importance in the risk assessment.

CAUSATIVE AGENTS AND EXPOSURE ASSESSMENT

Although the association between moisture and microbial growth and adverse health effects has been well shown in a number of epidemiological studies, the understanding of causal relationships between various exposing agents and the health effects are far from clear. The health effects probably have different causative agents and they may develop via different mechanistic pathways. At present, a number of different parameters can be used as surrogates of the moisture and mold-related exposure.

Since the fungal growth, mold, is the visible phenomenon that is observed in moisture damaged situations, fungal spores have been the primary candidates for being the causal factors of respiratory disorders. In the present literature, however, there is no conclusive evidence that increased concentrations of fungi in indoor air would be the actual cause for the symptoms (Verhoeff and Burge, 1997). The indoor concentrations of fungi in moisture damaged environments have been shown to be somewhat higher than in normal

conditions (Hyvärinen *et al.*, 1993, DeKoster and Thorne,1995) but the concentrations are usually far from those in outdoor air during the frost-free seasons. On the other hand, the spatial and temporal variation of indoor fungal concentrations is remarkable, and the concentration distributions of moldy and normal indoor environments are largely overlapping (Hyvärinen *et al.*, 2001).

In some cases, the source strength of a microbial growth in the structures is high enough to produce a notable increase in indoor concentrations of microbes, but in other occasions the levels remain low even when visible mold is present (Miller *et al.*, 2000). Concentrations of viable fungi under 100 cfu/m³ can be considered "low" and those over 1000 cfu/m³ can be regarded as "high". Evidently, measurements of viable fungi can be used in showing unusual microbial conditions in a building, but the concentrations as such are only rough surrogates of actual exposure and thus poor tools in systematic risk assessment.

The microbial flora of the indoor environment is usually determined from samples of viable fungi or from house dust samples. Apart from the normal mycoflora of indoor air, which consists mainly of outdoor air genera such as *Penicillium*, *Cladosporium*, *Aspergillus*, yeasts and non-sporing isolates, unusual genera or species may occur, albeit the diversity of indoor fungi is remarkable (Miller *et al.*, 2000). However, indoor air observations of the genera or species that typically grow on moisture damaged building materials, probably indicate the presence of such damage. Examples of such fungi are *Aspergillus versicolor*, *Aspergillus penicillioides*, *Stachybotrys chartarum*, *Chaetomium sp.*, *Fusarium sp*, *Trichoderma sp.*, *Acremonium sp*, and among bacteria, streptomycetes.

Concentrations of viable fungi in indoor air correlate poorly with health effects (Verhoeff and Burge, 1997) and have large temporal and spatial variation (Hyvärinen *et al.*, 2001). Furthermore, they only represent about 1% of the fungal particles actually present (Toivola *et al.*, 2002). Therefore, the microbial load of indoor environments has also been described with enzymatic methods or chemical markers of biomass. Structural components of microbial cells, such as endotoxins of gram negative bacteria, $1,3-\beta$ - glucans of fungi and some bacteria, and ergosterol of filamentous fungi are possible parameters to determine (Park *et al.*, 2001, Rylander 1997, Dharmage *et al.*, 2001). However, conclusive results on whether these parameters correlate with health effects in indoor situations have not been shown.

PCR techniques are rapidly developing, and applications of the new techniques for specific detection of microbes in indoor environments can soon be expected (Haugland *et al.*, 1999, Cruz-Perrez *et al.*, 2001, Rintala *et al.*, 2001). Based on the detection of genetic material of microbes, the techniques are independent on the viability of the microbes to be detected.

Volatile organic compounds, VOC, are involved in many occasions of biological or chemical decay induced by moisture. Some compounds have been suggested as being MVOC, microbial VOCs, although few of them are actually specific end products of microbial metabolism (Korpi *et al.*, 1998). The exact role of VOC exposures as causal agents of irritation symptoms is still obscure.

Microbial toxins are potential causative agents of several health effects. Toxins are produced by many microbial species occurring on moldy building materials, and many of these secondary metabolites are highly and acutely toxic. The occurrence of toxins in the spores has been shown (Croft *et al.*, 1986), and this may be the pathway how they end up in the indoor air and possibly into the human airways. However, it has not so far been shown that exposure to microbial toxins via airborne route takes place in indoor environments.

HAVE MICROBES BECOME MORE HARMFUL?

Through the history, man has empirically known that damp indoor conditions are unhealthy and harmful for the building. Traditional building practices and technologies have always aimed at keeping the building and indoor conditions dry, which has led to different solutions in different climates. In modern buildings, moisture control is a complex and demanding challenge. Modern building technology and material development have provided new ecological niches for microbes which may readily exploit the faults in moisture control. Microbes and their metabolic potential has always been there, it is the conditions in the building that have changed. Part of the control of moisture and mold should be regular maintenance which is often neglected in a pursuit to cost-effective use of buildings. Microbes, always showing remarkable adaptation ability and metabolic potential, take the chance whenever we let them.

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