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Microbiology on *Indoor Air '99* – What is New and Interesting? An Overview of Selected Papers Presented in Edinburgh, August, 1999

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Abstract A multidisciplinary approach to microbiological implications of indoor air is fruitful for research as well as management of health and building problems. The Finnish and the Danish mold programs are examples of such productive collaborative studies. Dust samples taken from classrooms in schools where occupants complain of building-related symptoms (BRS) demonstrated an inflammatory potential *in vitro*, measured as a release of cytokine interleukin (IL)-8. An increase of the metabolite NO and liberation of tumor necrosis factor (TNF)-α and other cytokines during exposure were obtained *in vivo*, was presented based on these programs and on epidemiological studies on residential fungal contamination and health conducted in Canada and The Netherlands. New methods for assessing fungal exposure are PCA analysis for the toxigenic mold *Stachybotrys chartarum* and EPS-*Asp/Pen* for detecting of *Aspergillus* and *Penicillium* in dust. Based on a limited data set it is shown that emission rates of fungal spores are inversely proportional to relative humidity (RH), directly related to flow rate and to surface loading. Poor maintenance, risk constructions and risk materials are described in several studies as the main causes of water damage in buildings.

Key words Water damage; *Stachybotrys*; Allergens; Toxic molds; Cytokines; EPS-*Asp/Pen*.

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Introduction

Owing to an unexpectedly large amount of microbiological papers submitted to *Indoor Air '99*, Edinburgh, International Conference on Indoor Air Quality and Climate, it was decided to include a keynote presentation highlighting points of selected papers, mainly aimed at building professionals. After the conference, I was invited to prepare a paper for *Indoor Air* based on my oral presentation.

In research as well as in practical management of microbiology and indoor climate it may be rewarding to take the following three essential aspects into account: health, microbiology and building aspects (Figure 1).

Such a multidisciplinary approach to various problems of mold infested buildings can be rewarding because much scientific and practical progress in this area often emerges in the interaction between disciplines and in the interfaces between them.

The Finnish and the Danish mold programs (Internet: http://www.ktl.fi./sytty/ and http://www.sbi.dk/English/Research/eogi/moulds.htm are examples of the attempt to include research areas that takes a multidisciplinary approach.

Important Conferences

Two international conferences, which included the previously mentioned three aspects, were held in 1998 in the US. One was *"Molds and Children's Health"*, a meeting partly sponsored by EPA and reported in Volume 107 of Environmental Health Perspectives Supplements (1999). Another was the "Third International Conference on Bioaerosols, Fungi and Mycotoxins – Health Effects, Assessment, Prevention & Control" held 23–25, 1998 in Saratoga Springs, NY, USA. The results were published in a book, which also included the proceedings from the meeting (Johanning, 1999).

Important Health Aspects

Residential Fungal Contamination and Health

Important results can be noted from the Canadian "Wallaceburg Study", which demonstrated that associ-

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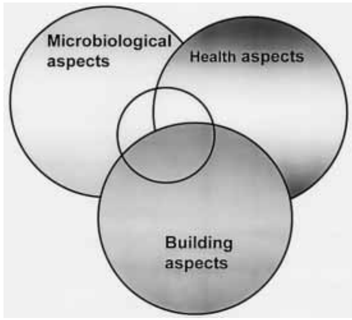

Fig. 1 The multidisciplinary concept

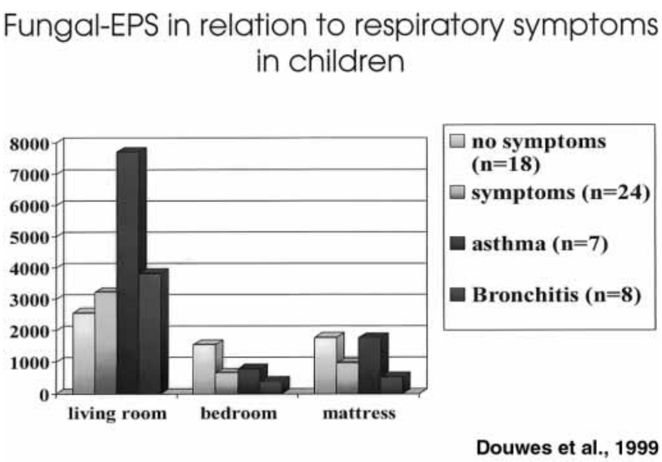

Fig. 2 Fungal-EPS in relation to respiratory symptoms in children (Douves et al., 1999)

ation between residential fungal contamination and symptoms is not confounded by house dust mites, bacterial endotoxin or other known disease-causing agents (Dales and Miller, 1999). Furthermore results from the study showed an increased number of lymphocytes suggesting that fungal residential infestation leads to chronic stimulation of children's lymphocytes (Dales et al., 1998).

In a Dutch epidemiological study, the group in Wageningen demonstrated that children with chronic respiratory symptoms were found to have higher levels of extra cellular polysaccharides from the mold genera *Aspergillus* and *Penicillium* (EPS-*Asp/Pen*) in their living room floor dust in comparison with children without respiratory symptoms, comparing high versus low exposure (Figure 2). A subgroup of children with asthma had even higher levels of EPS-*Asp/Pen* in their floor dust (Douves et al., 1999). EPS-*Asp/Pen* do not function as allergens or non-specific pro-inflammatory agents, but appear to be highly specific markers indicating presence of *Penicillium* and *Aspergillus*.

As *P. chrysogenum* and *A. versicolor* are some of the most frequently obtained species from water damaged building materials in the Nordic countries (Gravesen et al., 1998; Nielsen et al., 1999), this analysis seems to be an important tool for the assessment of fungal exposure, if practically applicable.

Another promising exposure study from the same group identifies an association between concentration of β(1,3)-glucans (non-allergenic, pro-inflammatory cell wall components, which may indicate the presence of fungi) in living-room floor dust and increased peak-flow variability in atopic children with chronic respiratory symptoms, when adjusted for the effects of house dust mite allergens, carpets and the presence of pets (Figure 3) (Brunekreef et al., 1999).

Fungal Infestation in Schools and Health

Complaints following occupancy in water damaged schools have been increasing over the past decade. It is suggested that the altered architectural and construction habits towards lower costs, especially from the 1960s and the 1970s, combined with cheaper materials, poor workmanship and poor maintenance are responsible for the many water damaged schools today in some countries (e.g. Denmark and Finland).

A crucial, but complicated and complex task is to give threshold values for the extent of mold growth on building materials that causes adverse health effects. Establishment of such guidelines would provide a useful tool for professional assessment of a strategy for intervention. The presence of a mold-infested surface of "xm^2" is, however, at best a crude proxy as long as we remain ignorant of many other health-related aspects of molds. These are, for example, emission of spores from growth on reverse surfaces to the air, the role of microbial volatiles, dose of mycotoxins produced on building material and liberated to the ambient air with spores or dust as vehicles, and the impact of species composition of the moldy spots.

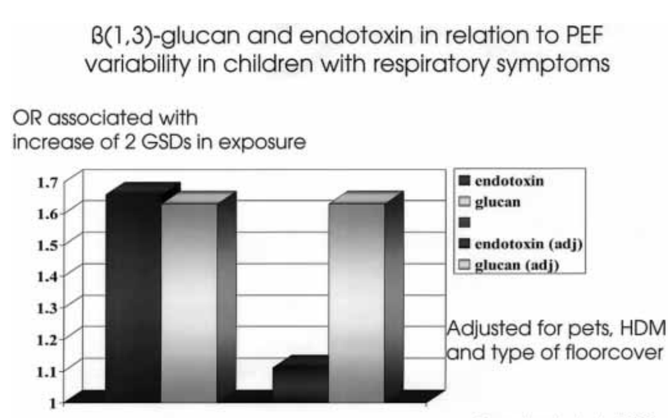

Fig. 3 β(1,3)-glucan and endotoxin in relation to PEF variability in children with respiratory symptoms (Brunekreef et al., 1999) PEF: Peak Expiratory Flow

A Danish epidemiological study in 75 schools was conducted in Copenhagen, and showed a statistically significant association with exposure to $\geq=0.25$ m^2 mold infested building material per classroom and building-related symptoms (BRS) among Danish female occupants. This corresponds well with Canadian observations, which demonstrated an association between BRS and areas of around 0.50 m^2 (Miller, personal communication). These observations are preliminary and should be treated with certain reservations until further data are available. The reason to be reluctant to introduce such values is that soon after their establishment they may be used as "golden standards" in occupational and environmental hygiene practice without any critical restrictions.

The Danish study showed moreover that dust obtained from classrooms in which the occupants reported BRS possessed an inflammatory potential. This was demonstrated as release of a higher concentration of the cytokine interleukin (IL)-8 from human lung cells (an A 549 BAL cell line) after provocation with the dust *in vitro* (Meyer et al., 1999). Cytokines are important mediator substances secreted by different blood cells as part of the immune response to harmful exposure.

Similar, and convincing, responses are measured in nasal fluids from the teaching staff at a moldy Finnish school. An increase of the metabolite NO and liberation of tumor necrosis factor (TNF)-α and other cytokines during exposure were obtained *in vivo* (Hirvonen et al., 1999).

These observations represent steady progress towards a better understanding of the underlying mechanisms behind the non-immunoglobulin E (IgE) mediated inflammatory response following exposure to non-infectious fungi.

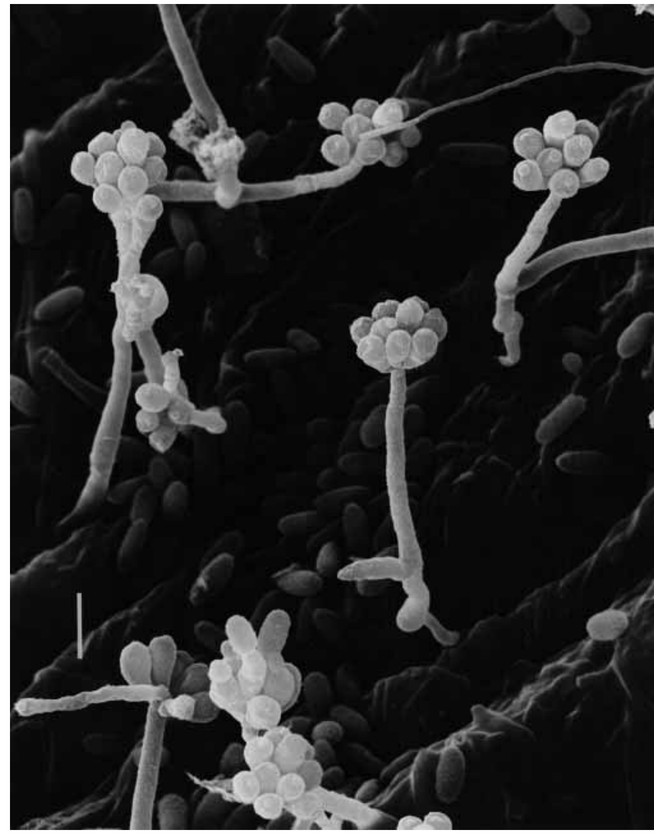

Fig. 4 SEM picture of *Stachobotrys chartarum* an important member of "the damp building associated funga". Magnification 2000×. Photo: Robert A. Samson (Gravesen et al., 1994)

Important Microbiological Aspects

Mold spores are ubiquitous, and constantly elevated humidity (>80% relative humidity (RH), or w_a>0.8) on the surface or inside a given building material containing organic components will inevitably lead to microbial growth and subsequent alteration of the structure of the infested materials (Figure 4).

"The Associated Funga"

In countries such as Canada, Denmark and Finland, studies are being conducted to establish and describe the molds contaminating humid building materials.

A prerequisite for establishing more comprehensive scientific evidence on the causal adverse health effects and the materials at risk of infestation is to generate more knowledge of "*the associated funga*", which infest water damaged building materials. In this context, the word "associated" is used to describe the actual genera and species of fungi connected with this particular ecological niche: water damaged building material.

The word "*Funga*" is a newly established concept, since fungi now have their own kingdom corresponding to the plant and animal kingdoms (the "*flora*" and the "*fauna*"). Characterization of the associated funga makes it possible to predict possible exposure to allergens and mycotoxins produced in mold-infested buildings. It is important to study demand of the individual fungal species for moisture of the building materials. Furthermore, investigations of resistance of different building materials and structures to mold infestation are important in order to avoid risk materials and risk constructions. This should be a practical planning tool for engineers and architects when designing buildings.

A mold species can unambiguously be characterized and given a name based on morphology, profile of mycotoxins and other biologically active metabolites in combination with a few physiological data. The experimental data behind the name of the species may predict the potential for growth and production of meta-

Table 1 Example of the associated funga of water damaged building materials

	allergens	mycotox
Penicillium chrysog.	+	no toxins
Aspergillus vercicolor	+	sterigmatocystins
Chaetomium spp.	+	chaetoglobosins
Ulocladium oudemansii	+	none
Stachybotrys chartarum	+	trichothecenes

(Gravesen et al., 1999)

bolites under field conditions. Adequate handling of practical problems with moldy buildings such as health implications, extent and mode of renovation and preventive measures depends on knowledge of these features.

Table 1 shows the five most frequently occurring mold species on 72 samples of building materials from schools in Denmark with substantial mold infestation (Gravesen et al., 1999)

The building associated funga differs from the airborne and dust bound one, although the ubiquitous species will to some degree occur in all of the three habitats.

One of the significant molds, frequently isolated from wet plaster boards, *Stachybotrys chartarum* is reported to cause health problems in countries where toxic strains of this mold have infested building materials as seen in USA after flooding episodes (Dearborn et al., 1999).

Allergenic Fungi

Since the wall of all fungal spores and hyphae contain protein it should be taken into consideration that all fungi are potential inducers of IgE-mediated allergy in atopic persons following sufficiently high exposure levels of fungal particulate. The previously mentioned mold species are all known to cause allergic reactions in such genetically predisposed atopic persons after inhalation (Gravesen et al., 1994).

Toxigenic Fungi

Previous studies on molds frequently present indoors and outdoors, such as *Alternaria* and *Cladosporium*, have been aimed mainly at the allergenic effects and characterization of allergens. Current investigations deal with the health implication of exposure to both allergens (protein) and fungal metabolites. Additional knowledge has, however, been acquired about the potentially negative role of inhalative *non-volatile* fungal secondary metabolites present in fungal particulate matter. Recent studies have revealed that different molds growing on building materials are able to produce substantial amounts of substances with adverse health effects other than a specific allergy. Some of these studies have demonstrated fungal metabolites such as *alternariols, chaetoglobosins, mycophenolic acid, satratoxins*, and *sterigmatocystins* detected in the buildings with mold-infested building materials. These are mycotoxins with potential dermatoxic, immunomodulating and carcinogenic effects among others.

Mycotoxins are "natural products (secondary metabolites) from molds which initiate a toxic response in vertebrates, when induced in small concentrations through a natural orifice, i. e. the mouth, the respiratory system or the skin" (Gravesen et al., 1994). Secondary metabolites are: "Extrovert, chemical differentiation products produced by living organisms" (Frisvad, 1998). As work is constantly progressing to screen fungal secondary metabolites for beneficial effects such as the effects of penicillin on bacteria and cyclosporin on the immune system (Gravesen et al., 1994), intensive studies are conducted to detect and characterize potentially harmful metabolites produced *in situ* in building materials.

For four of the previously mentioned most frequently occurring mold species in water damaged buildings, production of secondary metabolites on naturally infested building materials has been demonstrated: *Penicillium chrysogenum* produces the metabolites meleagrin and chrysogin, which have no known toxic effect (Nielsen, 1999).

Aspergillus versicolor produces the mycotoxins sterigmatocystin and 5-methoxy-sterigmatocystin (up to 20 and 7 $\mu g/cm^2$ of infested surface of a building material (Nielsen and Gravesen, 1998). Sterigmatocystin is a precursor of aflatoxin, the most carcinogenic toxin of biological origin known.

Stachybotrys chartarum may produce the macrocyclic trichothecenes satratoxin H&G (20–200 ng/cm^2 building material). In buildings low as well as high toxic strains can be isolated. A newly developed polymerase chain reaction (PCR) method makes it possible quickly to distinguish between high and low toxic strains (Land and Must, 1999), which may facilitate decisions on the degree and urgency of remedial measures. Further the mycotoxins roridin E, verrucarin J&B, stachybotrylactones, stachybotrylactams (Land and Must, 1999; Nikulin et al., 1994; Johanning et al., 1996; Nielsen and Gravesen, 1998) have been demonstrated in buildings infested with *S. chartarum*. This fungus is currently in focus due to reports of adverse health effects caused by *S. chartarum* (Croft et al., 1986; Etzel et al., 1998). Especially a cluster of cases of nose bleeding, blood spitting and pulmonary hemorrhage (lung bleeding among infants) following a flooding with subsequent residential infestation and high exposure to *S. chartarum* in Ohio, Cleveland has been intensely discussed (Dearborn et al., 1999). *Chaetomium globosum* produces the mycotoxins chaetoglobosin A and C (up to 50 and 7 μg/cm^2 building material) and more than ten unknown secondary metabolites (Nielsen et al., 1999).

Concerning the health implications of mycotoxins, further knowledge is needed in the following areas:

- Dose/response relationship following exposure in buildings.
- The relative toxic effect of combinations of toxins – antagonistic or synergistic.
- Possible changes in reactivity due to inflammatory processes, maybe leading to hyper-reactivity.
- Finally, and maybe the most crucial point: Valid clinical and routine laboratory tests for effects, like the established routine tests used for diagnosing a specific allergy.

Liberation and Dispersal of Fungal Spores

Doing professional assessments of water damaged buildings, adverse symptoms such as itching and burning skin, itching eyes, headache or even tightness of the chest are quite often experienced by technicians during a walk-through. Still we do not know much about emission of spores and volatiles in moldy buildings.

In a newly conducted study, emission rates of fungal spores for a limited data set are determined to be the following:

- Inversely proportional to RH
- Directly related to flow rate
- Directly related to surface loading (Foarde et al. 1999).

Methods for Assessing Exposure to Fungal Components

Even though our knowledge of the exposure route – "From wall to nose" – is restricted, detection methods for assessing exposure are needed. Preferably they should be standardized, cheap, simple and rapid. A comprehensive overview of new detection methods for assessing exposure to components of microbial origin is given by Dillon et al. (1999).

Non-Pathogenic Bacteria on Building Material – An Upcoming Issue?

Other interesting microbiological aspects was detection of non-pathogenic bacteria on building materials as potentially harmful agents, such as atypical strains of the genus *Mycobacterium* and of the species *Streptomyces griseus*, which induce toxic reactions and inflammatory cytokines *in vitro* (Mikkola et al., 1999; Peltola et al., 1999). Furthermore *Bacillus cereus* isolated from settled dust and water damaged building materials produces toxic substances (Andersson et al., 1998).

Important Building Aspects

Another observation in the "Wallaceberg Study, states that visible mold spots are no good predictors of degree of infestation, since much mold growth is revealed in hidden locations after opening suspected constructions (Lawton et al., 1998). This is an observation often repeated in the Nordic countries as shown in Figure 5.

Schools and Water Damage

Water damaged schools present a substantial problem in Finland and Denmark and similar problems have been reported in several other countries. In a Finnish study, it was demonstrated that moisture indeed increases the risk of respiratory symptoms and infections in schools and kindergartens (Koskinen, 1999). The main reasons for moisture damage in Finnish schools are difficult moisture conditions on the building site, poor ventilation and water leaks. Fortunately intervention with remedial measures in some of the moldy Finnish schools demonstrate a decrease in BRS among the schoolchildren and the teaching staff (Haverinen et al., 199).

Main reasons for water damage in Danish schools are water leakage through flat roofs, rising damp and defective pipes. Materials most susceptible to mold infestation are *wet* organic materials containing cellulose such as wooden material, wallpaper, glue, chip boards and plaster boards (Gravesen et al., 1999).

Conclusion

Handling the complex problems in buildings with water damage and subsequent adverse health reactions

Fig. 5 Engineer working on a waterdamaged school detecting heavy mold infestation on the reverse side of the building material. Note the protection mask. Photo: Kristian Fog Nielsen

requires the good professional judgement, so allow me to introduce the ideal co-worker, the "fungitective", who should possess the following capacities:

- A sound knowledge of building physics and hydrogeology, building technology and mold detection methods
- A special understanding of moisture and moisture calculations
- A basic knowledge of HVAC technology, building materials, chemistry, microbiology and medicine
- A critical approach to the measured results
- No preconceived ideas
- The ability to distinguish between *knowledge, belief* and *guesswork*
- And know his own limitations (Samuelson, 1993).

It would seem obvious, that such a person does not exist and that neither physicians, biologists nor engineers are able to handle these problems alone. So what happens during interaction and collaboration between the three disciplines often gives the most dynamic approach to research as well as to practical solutions.

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