

PART II:

ASSESSMENT & REMEDIATION

Associations between ventilation and mycological parameters in homes of children with respiratory problems

Hans Schleibinger, Daniel Aubin, Doyun Won, Wenping Yang, Denis Gauvin, Pierre Lajoie

The background of this contribution is the research into a healthy ventilation rate, especially for vulnerable parts of the population. This paper describes the results obtained during a randomized intervention study investigating the impact of ventilation rates on a wide range of indoor air quality (IAQ) parameters and the respiratory health of asthmatic children in Québec City, Qc, Canada. Following the ventilation intervention there was a marked decrease in the concentrations of a number of IAQ relevant parameters including mold spores demonstrating that ventilation interventions are effective at improving IAQ.

The Penetration of Mold Into Fibrous HVAC Insulation Makes Cleaning Impossible

Thomas G. Rand and Phil Morey

Fibrous insulation is often installed along the airstream surfaces of air handling units and air supply ducts for thermal and acoustical purposes. Glass fibers in new fiberboard and new fibrous liners are characterized by an apparent absence of dirt and mold growth. Dirt and dusts that accumulate on or within fibrous insulation are hydrophilic. During the air-conditioning process the relative humidity downstream

of cooling coils can consistently approach 100% and mold spores present on dirt accretions can germinate and grow. In some cases, the airstream surface of the insulation becomes covered with microfungi. In this study, we examined samples of new and mold contaminated airstream insulation, including moldy samples archived in dry storage for 18 yrs., using differential interference (DIF) and low vacuum scanning electron microscopy (SEM). None of the new insulation samples showed evidence of mold contamination. However, abundant hyphae, conidiophores, and spores were present on the mold colonized insulation surfaces, as well as in porosities several millimeters below the liner surface. Archived samples also contained hyphae, conidiophores, and spores, and although mold structures were sometimes melanized, they were still easily recognizable on and in the glass fiber matrix, despite their time in storage. As mold structures were on and around subsurface glass fibers, we then tested the hypothesis that attempts at physical cleaning would not be successful in removing embedded mold. To test this hypothesis, moldy, non-archived fiberboard was thoroughly vacuum-cleaned so that mold encrustation on the liner surface was visually removed. DIF and SEM examination of fiberboard after cleaning showed that subsurface hyphae, conidiophores, and spores were still present on and around glass fibers. It was concluded that vacuum cleaning of moldy fiberboard does not remove mold embedded in the fibrous matrix. Collectively, this study shows that moldy HVAC insulation cannot be physically cleaned and should be discarded.

Past, Presence and Future of Immunoassays for Mycotoxin Testing

Erwin Maertlbauer

I like to give you some information about immunoassay for mycotoxins. I will present a short history of mycotoxins so you better understand the history of immunoassays for mycotoxins. We do not know very much about mycotoxins problems in the past centuries but I guess that during that time, in the medieval ages, insulation of houses was not considered to be a major issue. We are certain, that ergotism was an important cause for thousands of victims in these ages and later on. You may have seen the famous painting by Pieter Bruegel illustrating the possible effects of intoxications shown in the picture with the title “the beggars”.

Health risks of surface disinfection in households with special consideration on quaternary ammonium compounds (QACs)

Axel Kramer, Harald Below and Ojan Assadian

Due to their antimicrobial mode of action the use of disinfectants requires a careful risk benefit assessment in order to minimize potential side effects on humans and in the environment as far as possible. Particularly the regular use of household disinfectants needs careful assessment, as the risk of contracting infection in household is less frequent than in a health care environment, because pathogen organisms are not prevalent in such high frequencies, and the immune status of most household residents is not decreased when compared to patients. Additionally, using disinfectants in household may be associated with unwanted health risks, such as allergy, sensitization, or intoxication. Therefore, risk assessment in presence of reduced risk for infection and an increased risk for other health affecting side effects may result in different conclusions for health care settings or households.

If, after careful consideration of a feasible indication or necessity for e.g. cleaning and surface disinfection of a household area with fungal growth, is given, selection and choice of a suitable disinfectant is imperative. Because of toxicological reasons, aldehydes are to be rejected. Quaternary ammonium compounds (QACs) are considered to show a low-level antimicrobial efficacy and so far, are considered harmless. Therefore, QACs are most probably the most widely used antimicrobial compounds in households in Europe and the US. Based on a critical risk benefit assessment and first reports of adverse effects after chronic use in household cases, it may be assumed that a long-term use of QACs on surfaces or even a single nebulization in households is associated with occupational and household health risks. To clarify this association, further research needs to be conducted.

There are, however, a number of alternatives to QACs. For repeated use on smaller surface areas, alcohols may be used. On larger surfaces, surface disinfectants on basis of organic carbonic acids or oxidants are suitable. Instead of using products on basis of QACs, various H₂O₂ formulations without environmental persistence (if the dispensable critical catalysator silver is not used) are to be preferred.

Toxicity study of field samples from water damaged houses in flooded areas in Poland

Magdalena Twarużek, Jan Grajewski, Manfred Gareis

This presentation summarizes the results of air quality investigations conducted in flooded homes in Poland. During the last decades there have been periods of significant draughts and floods in certain areas of Poland. These natural disasters may result in additional health threats to the inhabitants because of the major moisture damage and mold growth inside residential houses.

Flood simulations performed by the British Hadley Centre in Bracknell have shown that in the coming years large areas of Poland may be increasingly threatened by flooding. Other European countries may be also affected by this. The evidence for these threats is strengthened by the disaster caused by the flooding of the Oder and Vistula rivers, and recently also in Germany and the Czech Republic. The flooding of the Oder in 1997 resulted in the death of 114 people in 2592 villages and about half a million hectares of farm land were under water. The total loss was estimated at 6.5 billion euros (Holdys, 2004).

The environmental conditions caused by flooding in these areas resulted in large scale mold growth and contamination in buildings and resulted in an immediate threat to the health of the residents. This fact is confirmed by the example of the Wrocław - Brochów geographic area. Temporary residential housing had to be provided by the government. Many tenants could not return to their former homes because the interior mold growth and the likely adverse health effects in their homes prevented them from returning (Twarużek, 2005). The General Office of Building Control provided these temporary homes, however they were often in poor physical condition, and most importantly, many design defects were obvious: lack of proper heating, inadequate ventilation and damp soil, which all resulted in stains, moisture and interior mold growth (Janińska, 2002).

In 2002, another large scale flooding occurred in Poland and damaged buildings and houses in the north and south of Poland. Due to the increasing research interest in the fungal pathology of different species in the indoor environment, an effort was made to study the mycology and mycotoxins analysis of air and building partitions in the houses of the flooded areas. Earlier reports by other authors (Gareis 1994, Gareis et al., 1998; Johanning et al., 1998) were a motivation to conduct the following examinations.

Mycotoxin screening of indoor environments in sentinel health investigations

Eckardt Johanning, Manfred Gareis

In environmental and occupational studies of bioaerosols risk assessment is typically based solely on fungal identification and quantification. However, specific health outcomes are often poorly correlated with these parameters. This may be in part due to biological fungal air contaminants that have different toxic properties. Traditional in-vitro chemical laboratory analyses of fungi have practical limitations. In-vivo toxicity screening appears preferential to explore complex health reactions reported by exposed patients. In these sentinel health effect studies, we compared conventional fungal identification methods with a screening test for mycotoxins (cytotoxicity of mycotoxins evaluated by the MTT-cell culture assay) and EIA quantification of trichothecenes designed for detection of Roridin A and other macrocyclic trichothecenes. High (24h) volume air sampling to collect air-borne particles (n=225) was conducted in homes and work places of patients (n=115) with environmental symptomatology and visible fungal indoor growth. The crude extracts of approximately two thirds of the air samples showed mild to high toxicity in the MTT cytotoxicity assay and 19 % of n=214 samples had Roridin A results of >10 ng/g. Among all the fungi identified, there was only a weak association of viable *Stachybotrys* fungi and Roridin but not with other fungi. In conclusion, traditional fungal identification methods in bioaerosols exposure studies appear to be a poor predictor of toxicity without the use of the effect-based toxicity bioassay to assess and confirm toxicity. The MTT cell culture cleavage assay is an effect-based screening test which has appears to be quick and easy method. It facilitates evaluations of the biological activity of many different mycotoxins. It may provide a useful tool for the testing of a large variety of sample materials, including indoor air contaminants.

A Comparison of two Sampling Media (MEA and DG18) for Environmental Viable Molds

Sirkku Häkkinen

Often the evaluation of fungal damage in buildings is carried out by culturing samples on different kind of growth media in laboratory conditions. This paper gives information about how choice of culture media does affect on the results you get, and what kind of differences are there between the two most often used mould culture media in indoor environmental studies. In the study a comparison of the culture results of 200 air samples and 200 material samples cultured both on Malt

Extract agar and Dichloran Glycerol 18 agar was carried out. The mean total CFU counts and number of taxa on each media was almost the same. Although there were there were no significant differences between the two media in mean total CFU - counts /m³ or /g in some samples the interpretation of the culture results would be different if only one type of culture medium was used. The biggest differences between the two media, was in the species composition: the xerophilic species were almost absent from MEA.

Evaluation strategy in damp buildings and use of infrared thermography

Yves Frenette

The Public Health Department in Montreal (PHDM) receives a hundred of notifications per year for health problems related to housing buildings. According to the Québec Public health law, physicians and municipalities for instance have to notify potential health threats related to biological, chemical and physical contaminants. Those mainly concern tenants living in multiplex buildings, but also schools, nursery homes, hospitals and other types of buildings.

Apart from these investigations, the PHDM supports city district inspectors in the application of the By-law concerning the sanitation and maintenance of dwelling, as well as local health professionals, provides training on indoor air quality problems and is implicated in research programs (Vg: Respiratory health of children, Effectiveness of home-based environmental interventions).

As part of its investigations, the environmental hygienist must quickly determine if the building is probably contaminated by molds and represents a risk to the health of the occupants. The aim of this article is to explain the strategy used to identify the conditions favouring molds growth and all the methods used in the most effective way, including the infrared thermography. We also present the results of the investigations we have performed on a sample of building apartments.

Prevalence of mold observations in European housing stock

Ulla Haverinen-Shaughnessy

An assessment of the prevalence of mold problems in European housing stock was carried out. The assessment relies on recent studies, taking into account regional and climatic differences, as well as differences in study design, methodology, and

definitions. It is based on general indicators of mold in dwellings, such as visible mold or mildew on surfaces. Similar indicators have been commonly used in an absence of more specific microbial markers of exposure. Epidemiological studies have also used similar indicators to estimate exposure-response relationships, associating presence of mold with health effects. Data were available from 22 European countries. Median prevalence of mold problems was 15.5%, and weighted mean prevalence was 10.3%. In addition to survey factors, climate characteristics (mainly temperature) appeared to influence on the prevalence values. Significant (up to 18%) differences were observed for prevalence of mold problems depending on survey factors and climate.

Indoor Molds and Respiratory Hypersensitivity: A Comparison of Selected Molds and House Dust Mite Induced Responses in a Mouse Model

Marsha D W Ward, Yong Joo Chung, Lisa B Copeland, Don Doerfler and Stephen Vesper

Molds are ubiquitous in the environment and exposures to molds contribute to various human diseases. Damp/moldy environments have been associated with asthma exacerbation, but mold's role in allergic asthma induction is less clear. The molds selected for these studies are commonly found indoors, associated with water damaged buildings and/or sick building syndrome. The studies objectives were to 1) elucidate the association between specific molds and allergy/asthma and 2) assess the relative allergenicity of these molds by comparing responses to those induced by house dust mite (HDM) using a mouse model.

Female BALB/c mice received 1 or 4 exposures by intratracheal aspiration of 0-80 µg of mold extract or HDM. Airway responses (PenH) to methacholine (Mch) challenge were measured on day 1. Serum and bronchoalveolar lavage fluid (BALF) were collected on day 2 after the final exposure. Serum extract-specific IgE and BALF inflammatory cell counts are presented.

Responses to mold extract exposure varied among the molds but multiple exposures were required to induce significant increases in extract-specific IgE and elevated levels of BALF eosinophil counts. To achieve similar results to those induced by HDM in the extract-specific IgE assay required 1.5X more *Scopulariopsis brevicaulis* and 2.25X more *Epicoccum nigrum*. However, *Penicillium crustosum* group did not induce

a significant extract-specific IgE response at any dose level. Multiple extract exposures also induced significant change in airflow (PenH) following Mch challenge.

The data suggest the capacity of molds to induce allergic responses varies. It also suggests there are threshold doses for allergic sensitization.

What does the development of fungal systematics mean to DNA-based methods for indoor mold investigations?

De-Wei Li and Chin S. Yang

In the last decade, DNA sequence technology has greatly advanced our understanding of phylogenetic relationships of fungi. This article reviewed the literature on the latest development of fungal systematic and its impact on DNA-based analytical methods currently used in indoor mold investigation. Among many studies, *Cladosporium cladosporioides* s.l. was redelineated and 22 species had been newly described from it. This study will have a significant impact on indoor fungi investigations using either morphological methods or DNA-based methods. *Stachybotrys chartarum* collected indoors was found to have two distinct phylogenetic clades which represent two different species. Current Quantitative Polymerase Chain Reaction or QPCR method is not able to differentiate *Stachybotrys chartarum*, *S. chlorohalonata*, and *S. yunnanensis*, and *S. cylindrospora* (non *S. eucylindrospora*). The biggest challenge of DNA sequence technology in mycological research is that up to 27% DNA sequence data deposited in GenBank are questionable or erroneous due to incorrect identification of the fungal materials. The conclusion is that DNA-based analytical methods, similar to morphology based methods, have showed great advantages but also have their limitations. Morphology and DNA-based analytical methods are supplementary. Indoor fungi investigators should understand the pros and cons of each test method and carefully choose the method of lab analysis which will best accomplish their objectives.

Case study: Determination of moisture damages on items of art in exhibitions by the use of microbial analysis

Judith Mueller and Urban Palmgren

The described case studies concerning the distinctive features and demands of investigations of mold growth on art and other objects of exhibition. The surfaces and materials used in this objects, pose a special challenge for sampling and analy-

sis. In the microbiological assessment of damages of art objects, the conservation of the value of the object, the search for the cause and the age determination of the damage and the minimization of the damage were the primary targets, as well as attending to the interests of museums, artists and insurances.

Investigation of effectiveness of mold disinfectants and chemicals on total cell numbers of mold on building materials

Judith Mueller and Urban Palmgren

In this investigation mold disinfectants H₂O (30%), H₂O₂ (5%) with hydroxy-acids (fruit acids) and isopropanol (70%) were tested on *Aspergillus versicolor* growth on wall paper pieces. The control treatment was H₂O. The aim of the study was to examine if the chemicals have an effect on the total cell count. The disinfectants had four incubation times: 0h, 2h, 24h and one week. The results have shown that the amount of reduction of the total cell count is not sufficient to replace the remediation of infected material with disinfection. In addition, the biochemical activity and the CFU have shown a habit of recovering after only one week, without nutrients and fluids available. This emphasizes the ineffectiveness of disinfectants to remove the biomass. Furthermore, the long term effect of mold disinfectants has not been investigated. In regard to preventive health protection, indoor mold contamination should be removed and replaced rather than treated by disinfectants.

Microbiological characterization of aerosols isolated from remote lakes in the Chilean Patagonia

Escalante G, León C, Campos V, Urrutia R and Mondaca MA.

The long range mobilization of biological material mainly bacteria and fungi (bioaerosols) has increased in recent years due to the effects of Climate Change (Moulin, Chiapello, 2006; Neff et al., 2008). The bioaerosols transport occurs from warm regions and lower latitudes of the planet to colder regions and higher latitudes, such as Patagonia area. According to this mechanism, most bioaerosols may accumulate in the Polar Regions, where low temperature would allow them to be deposited (De la Rosa, Mosso, Ullan, 2002).

The role of bioaerosols deposition into remote lake ecosystems is a potentially important process but not yet totally explored. The flow of airborne bacteria could be an important route of colonization of remote and pristine environments, with ecosystems that have no local anthropogenic influence, such as Patagonian lakes (Catalan et al., 2006). The risk of microorganisms dispersion to remote areas may have effects on the ecosystem of Patagonian lakes such as alteration of biogeochemical cycles, food webs and also can produce different diseases in plants, animals and even to the human beings (Griffin, 2007).

In Chile, there are few studies related to bioaerosols, so it is important to study the effects of the presence of allochthonous microorganisms in lake ecosystems, allowing a better understanding of the response of these ecosystems to the influence of external agents.

The aim of this study was to detect and characterize microorganisms in bioaerosols of remote lakes in the Chilean Patagonia (Alto Lake, Esponja Lake and Verde Lake).

Use of Culture and PCR Analysis in Mold Assessments

Philip R. Morey

Both PCR and culture analysis of collected samples can result in similar building assessment conclusions. However, both methodologies are subject to limitations. For example, the utility of PCR data in a mold assessment is limited by missing target taxa. Interpretation of PCR data is also problematic, as in the ERMI approach, when a taxon is misclassified as to its water indicator or phyloplane status. The use of culture data is limited by the longevity of spore culturability among species in aged samples. However, the most important aspect of a mold assessment is the physical inspection of the building and its HVAC system for evidence of dampness and biological growth. This study shows that the greatest limitation in mold assessments occurs when there is an over reliance placed on data obtained from sampling.

Factors promoting the exposure to bioaerosols among swiss crop workers

Hélène Niculita-Hirzel and Anne Oppliger

Agricultural workers are among the professional groups most at risk of developing acute or chronic respiratory problems. Despite this fact, the etiology of these

occupational diseases is poorly known, even in important sectors of agriculture such as the crops sector. A chronic exposure to multiple microorganisms, such as different bacterial and fungal species, has been proposed to be the cause of these multiple respiratory pathologies. Nevertheless, these microbial communities are still partially known. The aim of this study is to characterize all fungal species inhaled by the crops workers during different grain related activities and identify the abiotic and biotic factors that reduce the growth of the toxigenic, irritative or allergenic microbial species. Here, we are presenting the factors promoting the exposure to bioaerosols during different wheat related activities: harvesting, grain unloading, baling straw, the cleaning of harvesters and silos. Total dust has been quantified following NIOSH 0500 method. Reactive endotoxin activity has been determined with *Limulus* Amebocyte Lysate Assay. All molds have been identified by the pyrosequencing of ITS2 amplicons generated from bioaerosol.

The investigation of mold occurrence inside selected Durban homes.

Nkala BA, Jafta N and Gqaleni N

Introduction

Dust collected from indoor environments has been reported to contain biological pollutants such as pollen, spores, molds, bacteria, viruses, allergens, dust mites and epithelial cells. This study was aimed at assessing the presence of molds in house dust from selected Durban residences.

Methods

One hundred and five (105) house dust samples were obtained from households. The samples were taken from three surface areas namely; living room couches, bed mattresses, and carpets. Well documented methods were used for the isolation, identification and quantification of mold.

Results and conclusion

Among the isolated genera in all three surface areas, *Rhizopus spp.* and *Penicillium spp.* were widely distributed throughout in greater proportion. The overall highest colony forming units per gram of dust (CFU/g) for *Penicillium spp.* range: 3400 – 62316 CFU/g, was obtained from living room couches, followed by *Rhizopus spp.* (5200 – 15990 CFU/g). The mold results were compared with the South African Occupational Health and Safety Act (OHSA) 85 of 1993 as amended suggested guidelines of 1,000, 000 CFU/g. The findings of this study suggest that the molds in the homes studied were below the suggested guideline. However, this does not

imply that the indoor conditions are unsafe or hazardous. Instead, the findings act as an indicator of mold presence indoors. The type of airborne mold, its concentration and extent of exposure and the health status of the occupants of a building will determine the health effects.

Conserving our cultural heritage: the role of fungi in biodeterioration

Hanna Szczepanowska and A. Ralph Cavaliere

The objects of cultural heritage are composed of varied materials which can be affected by diverse microbial communities. The study of these complex and heterogeneous assemblies of materials and microorganisms require an inter- and multi-disciplinary approach. Development of a strategy towards prevention, mitigation of biodeterioration and removal of microorganisms, especially fungi begins with the understanding of the materials' fabric, assessment of causes behind the biodeterioration, and the context in which it occurs.

Three aspects of biodeterioration of cultural heritage are discussed: 1) the multitude of bio-agents' on cultural heritage materials, 2) fungal interaction with substrates, and 3) prevention and conservation of biodeteriorated artworks. The challenges of conservators' work in dealing with bio-degraded museum collections are discussed based on the case studies of biodeteriorated art on paper, exemplifying two types of fungal interaction with the substrate: 1) surface deposits of pigmented spores/conidia, and 2) pigmented fruiting structures embedded in the matrix of the substrate.

The microbial metabolites deteriorate the substrates on which they grow resulting in chemical and physical changes of the material bulk and surface, at times leading to structural weakening. We focused our studies on black stains which are prevalent on art rendered on paper, a subject that has received very little attention. Our techniques of analysis included three-dimensional topographic imaging and visualization, structural characterization and optical microscopy, scanning electron microscopy (SEM), and confocal laser scanning microscopy (CLSM).