Exposure to microbes in indoor environments and health

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Indoor microbes and health – two main types of adverse health effects

- Spread of pathogens
  - Role of air currents, contamination of surfaces, survival and infectivity (not at focus of this training course)

- Dampness, moisture and microbial growth associated with various adverse health effects
  - Association strong; causal links unclear
  - Exposing agents not quite clear
  - Good surrogates available
Dampness - mould - health

Moisture damage

Mold growth

Health effects
What is the exposure in damp/moisture damaged buildings?
Different meanings of “exposure”

- Epidemiological meaning: the factor that may be associated with health outcomes; is often a surrogate of actual exposure
- Toxicological meaning: the designed dose that are purposefully given to experimental animals/cells/tissues
- Exposure sciences: studying the exact character and behavior of the agent/factor people are exposed to
Exposure assessment in practical building investigations

- Measurement of actual exposure of humans not feasible
- In practice, assessment whether the occupants are exposed to agents possibly harmful to health
- Using well-tested surrogates gives an idea of the microbial status of the building
- Once moisture or mold problems indentified, it is known that it is a possible health risk
- remedial steps can be taken
Emissions from the harmful source to the indoor environment

- Particle emissions
  - Spores 1-20 um, fragments 10nm-1um
  - Spores may be viable or non-viable
  - Particles contain bioactive components
  - Particles may carry allergens, toxins, MVOCs
  - Particles from non-microbial material decay

- Volatile emissions, CO₂
  - Odors of mold, earch, cellar, fruit
Exposing agents in moldy buildings

- Fungal or bacterial spores, cells, their fragments
- Bioactive agents of microbial material
  - Allergens or beta glucans from fungi, endotoxin from bacteria
- Toxic metabolites from growing mold
  - Mycotoxins, bacterial toxins
- Volatile metabolites from growing mold
  - MVOC; odor of mold, cellar, earth
- Spores and other particles from dried mold
  - include toxins, allergens, other components

- Actual exposure consists of all these agents!
- Health importance of individual agents poorly known
Exposure assessment in science and practical building investigations

• Research on exposure must be done in order to reveal the causative agents of health effects
  – Helps to create basis and to develop many aspects of practical work

• Buildings must be investigated and remediated even now when causal links are not yet fully known
  – Good practices also learned by doing and seeing
Scientific literature - surrogates of mold exposure used in population studies:

- damp, dampness, damp spots, damp stains, wet/damp spots, condensation, window pane condensation, basement water damage, water damage, leaking, moisture stains
- visible mold, molds, mildew, mold growth, mold damage, fungal mold, stale odor, mold odor, silver fish/sow bugs
- Sometimes measurements of fungi, bacteria, biological particles or microbial components from indoor air, surfaces, house dust or from damaged materials
Indices of “dampness” or “mold” - a simplified summary

• Many different ways to express the exposure in question, end result generally the same:
• Dampness and the consequent mold is linked with building damage and adverse health effects
• This is a good rule of thumb!
Individual cases of building mold are complex and diverse

- “All happy families resemble one another, but each unhappy family is unhappy in its own way” (L. Tolstoy, Anna Karenina (1877))

- Also health outcomes are diverse
- Tailored study designs are needed for various purposes
- Building investigations: applying knowledge and experience for best possible assessment
Microbial concentrations in indoor air - what does science say?
Scientific evidence between airborne microbial concentrations and health is weak

- Few data on effects of air concentrations of fungi and bacteria on health
  - Airborne concentrations of microbes not a good measure of actual human exposure
- Concentrations vary in space and time
  - Exact assessment of microbial exposures is difficult, labor intensive, expensive
- Critical exposures are interactions of many agents
  - Simultaneous exposure to microbial particles, their components, microbial products (toxins and MVOC)
Some estimates of microbial concentrations indoors

- Concentrations <100 cfu/m³ often considered “low”,
- >1000 cfu/m³ indoors is often considered “high”
- Viable concentrations appr. 1% of total (Toivola et al 2002)
- Concentrations vary in time and space;
- Both in indoor and outdoor air
- Strongly dependent on climate, weather, season, location, activities
- Obviously, mere concentration measured in cfus is not the causal factor of health effects
- However, tells about the indoor air quality
Fungal concentrations are somewhat higher in homes with mold damage

Hyvärinen 2002
Hyvärinen et al. 2001
Air sampling – good for scientific studies?

- Based on present literature; single, short time samples for microbes in indoor air are no good.
- Either many samples or long-time integrated sampling.
- Measuring cfu/m$^3$ or total counts no good.
- A careful selection of parameters to be measured; e.g. QPCR for selected species.
Air sampling – is it for any practical use at all?

- Higher concentration in an indoor location indicates an indoor **source**
- Air sampling sometimes useful,
  - To show the exact room/location of the damage
  - To show airborne transport of microbial agents from space to space
- For result interpretation, always more than one sample
Quantitative assessment of microbes not enough – look at the species

- When moisture conditions change, the microbial conditions change
- -> altered species content of the environment
- Know the normal: *Penicillium*, *Aspergillus*, *Cladosporium*, yeasts
Examples of fungal genera found in infested building materials

<table>
<thead>
<tr>
<th>Acremonium</th>
<th>Gliocladium</th>
<th>Scopulariopsis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alternaria</td>
<td>Humicola</td>
<td>Sphaeropsidales</td>
</tr>
<tr>
<td>Aspergillus</td>
<td>Mucor</td>
<td>Stachybotrys</td>
</tr>
<tr>
<td>Aureobasidium</td>
<td>Oidiodendron</td>
<td>Torula</td>
</tr>
<tr>
<td>Botrytis</td>
<td>Paecilomyces</td>
<td>Trichoderma</td>
</tr>
<tr>
<td>Chaetomium</td>
<td>Penicillium</td>
<td>Tritirachium</td>
</tr>
<tr>
<td>Cladosporium</td>
<td>Phialophora</td>
<td>Ulocladium</td>
</tr>
<tr>
<td>Doratomyces</td>
<td>Phoma</td>
<td>Verticillium</td>
</tr>
<tr>
<td>Eurotium</td>
<td>Rhinocladiella</td>
<td>Wallemia</td>
</tr>
<tr>
<td>Fusarium</td>
<td>Rhizopus</td>
<td>Yeasts</td>
</tr>
<tr>
<td>Geomyces</td>
<td>Rhodotorula</td>
<td></td>
</tr>
</tbody>
</table>
Examples of bacterial genera found in moldy building materials

- Acinetobacter
- Agrobacterium
- Artrobacter
- Bacillus
- Brevibacterium
- Cellulomonas
- Clavibacter
- Corynebacterium
- Dietzia
- Flavobacterium
- Gordonia
- Methyllobacterium
- Microbacterium
- Nocardia
- Nocardiopsis
- Rhodococcus
- Spirillospora
- Streptomyces
- Thermomonospora
Microbial exposure assessment – problems and alternatives

- Concentrations of airborne microbes vary greatly
- Concentrations may be low even in damaged rooms
- For reasonable interpretation, several samples are needed -> higher costs
- In bulk samples the concentrations do not vary constantly
- Other than air samples easier to interpret
- Therefore, rather samples from materials or surfaces or house dust than from air
Other methods to assess microbial exposures

- Chemical markers for microbial communities:
  - Muramic acid for bacteria
  - 3-OH-fatty acids for gram negative bacteria
  - Ergosterol for fungi
  - Beta-glucan (fungi) or peptidoglycans (bacteria)

- DNA based methods
  - Microbial community analyses
  - QPCR for quantitation of specific microbes
House dust as a sample matrix for indoor microbes

- Most abundant fungi in house dust
  - *Asp/Pen/Paec* (median $5.44 \times 10^6$ cells/g)
  - *Aureobasidium pullulans* (median $4.35 \times 10^6$ cells/g)
  - Concentrations $10^2 - 10^4$ times higher than culture results
Penicillium brevicompactum (QPCR) in house dust vs. moisture damage

$p = 0.003$

0 = no moisture damage, 1 = moisture damage in one area, 2 = moisture damage in two areas, 3 = moisture damage in three or four areas in the house
qPCR / *T. viride/atroviride/koningii* vs. extent of moisture damage

$p=0.026$

Lignell et al., LAM 2008
Summary of house dust microbial community studies

- Fungal and bacterial diversity extensive in house dust
- Yeasts and basidiomycetes dominate the mycobiota
- Gram-positives dominate the bacterial flora
  - originate from human skin, gut etc.
- Remarkable seasonal variation, various sources can be observed
  - outdoor air, humans etc.
- Great variation between buildings; for bacteria mainly due to human individuals
- Effect of moisture damage not seen
Do DNA techniques solve the microbial measurements problems in practice?

- Fungal and bacterial communities in indoor environments are rich and diverse
- Indoor air is a mixture of particles from many sources;
- Pollutants from the mold growth are "a needle in the haystack"
- Finding the needle; knowing exactly what to look for
Presence of students in a classroom increases concentrations of airborne bacteria – muramic acid as a bacterial marker (Fox et al. 2005)

<table>
<thead>
<tr>
<th></th>
<th>Mur in dust/pmol mg(^{-1})</th>
<th>Mur in air pmol m(^{-3})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Occupied</td>
<td>Unoccupied</td>
</tr>
<tr>
<td>Occupied</td>
<td>18.2 (\pm) 9.6</td>
<td>0.14 (\pm) 0.10</td>
</tr>
<tr>
<td></td>
<td>8.9 (\pm) 4.4</td>
<td>3.47 (\pm) 1.69</td>
</tr>
<tr>
<td></td>
<td>17.1 (\pm) 14.3</td>
<td>4.71 (\pm) 2.44</td>
</tr>
<tr>
<td></td>
<td>Occupied</td>
<td>Unoccupied</td>
</tr>
<tr>
<td>Unoccupied</td>
<td>115.5 (\pm) 43.6</td>
<td>6.97 (\pm) 4.82</td>
</tr>
<tr>
<td></td>
<td>109.2 (\pm) 45.9</td>
<td>3.47 (\pm) 1.69</td>
</tr>
<tr>
<td></td>
<td>80.2 (\pm) 33.5</td>
<td>4.71 (\pm) 2.44</td>
</tr>
</tbody>
</table>
When “water damaged” buildings compared with “non-damaged” buildings

• Concentrations of airborne fungi slightly higher in damaged buildings
• Microbial flora different from normal
  – Indicators e.g.: *Stachybotrys*, *Aspergillus versicolor*, *A. penicillioides*, *A. fumigatus*, *Trichoderma*, *Chaetomium*, *Fusarium*, *Ulocladium*, *Acremonium*, *Streptomyces* (bacteria)
• Differences in MVOC, other parameters?
Summary of differences between index- and reference buildings
(Hyvärinen et al. 2001)

<table>
<thead>
<tr>
<th>Building characteristics</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture damage</td>
<td>yes</td>
</tr>
<tr>
<td>Visible mold</td>
<td>yes</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory symptoms</td>
<td>yes(^1)</td>
</tr>
<tr>
<td>Respiratory infections</td>
<td>yes(^1)</td>
</tr>
</tbody>
</table>

\(^1\)Husman et al. (1993), Koskinen et al. (1995)
### Summary of differences between index- and reference buildings
(Hyvärinen et al. 2001)

<table>
<thead>
<tr>
<th>Parameters of indoor air quality</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Airborne concentrations of viable fungi</td>
<td>Yes</td>
</tr>
<tr>
<td>Size distribution of viable fungi</td>
<td>Yes</td>
</tr>
<tr>
<td>Fungal composition of air</td>
<td>Yes</td>
</tr>
<tr>
<td>Airborne concentrations of viable bacteria</td>
<td>No</td>
</tr>
<tr>
<td>Concentrations of formaldehyde</td>
<td>No</td>
</tr>
<tr>
<td>Concentrations of TVOCs</td>
<td>Yes?</td>
</tr>
<tr>
<td>Concentrations in MVOC</td>
<td>No?</td>
</tr>
<tr>
<td>Indoor air temperature</td>
<td>Yes</td>
</tr>
<tr>
<td>Indoor air relative humidity</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Personal exposure to bioaerosols study (Toivola et al. 2002)

- Personal sampling for 2 x 24 hours
- Although not much difference in microbial counts, there was difference in biological activity (measured as IL-6 production of cells) of the samples
- Measuring the right thing...
Biological activity of the particle material collected on filters in relation to low or high personal microbial exposure (Roponen et al., Inhal Toxicol 2003;15:23-38)
Inflammatory markers in nasal lavage samples of occupants

- This might be an example of biomarker of exposure
- Limitation: individual variation also great
- Probably works best on group level
NO-production in nasal lavage fluid

Hirvonen et al., 1999
Health effects and dampness/mold

- several respiratory symptoms, irritation symptoms, general symptoms
- Symptoms mimick allergic symptoms, but not necessarily IgE-mediated
- asthma symptoms, onset of asthma
- Risk for other diseases
- Symptoms often disappear when elsewhere
Early exposure to farming environment protective from allergy

- Farming and rural children have less allergy than urban children (e.g. von Ehrenstein et al. 2000, Braun-Fahrländer et al. 1999, Riedler et al. 2000)
- Protective effect shown for endotoxin, EPS-\textit{Pen}/\textit{Asp} (Douwes et al. 2006), 1,3-\textit{ß}-glucan and dust (Gehring et al. 2007)
- Also contradictory findings
- Several birth-cohort studies going on
Why the paradox?

- Dampness-related mold harmful to health, but farming microbes protective from allergy
- No definitive explanations yet
- Hypothesis: the difference between the exposures in moldy buildings and farming environment is the toxin production that takes place in mold growth on moist building materials
Conclusions

- Links between dampness/mold and adverse health effects well documented
- Exact causal relationships not yet well known
- Actual exposures in a mold-problem indoor environment are complex and difficult to quantify
- The harmful source of exposure is the microbial growth in and on building structures
- Avoidance and control of exposure necessary
- A variety of good surrogates of exposure:
  - Indices of dampness, unusual microbial findings

Practical building investigations and remediations focus on the building and its indoor environment
Are there health-based guideline values for building microbes?

- Not possible to give health based TLVs or other numerical guideline values for biological particles
  - Causal links not known
  - Dose-response not known
- No help in deciding when the exposure "too high"
- For practical field work, guidance to interpret the results have been given
- Help to conclude, if concentrations and species are normal or not
Exposure may be intensive during remedial work

- Highest exposure during dismantling
- Containment and negative pressurizing of the renovation area
- Personal protection
- Good practices with contaminated waste