Exposure to microbes in indoor environments and health

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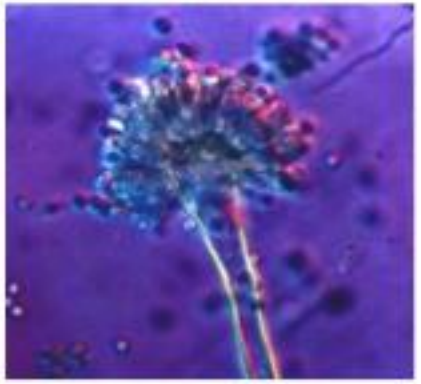
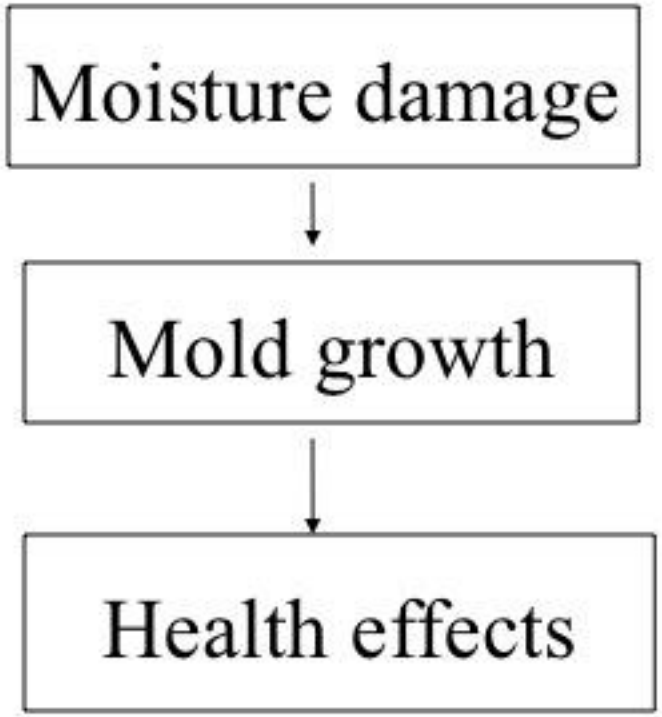
Indoor microbes and health – two main types of adverse health effects

- 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



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, CO_2
  - 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

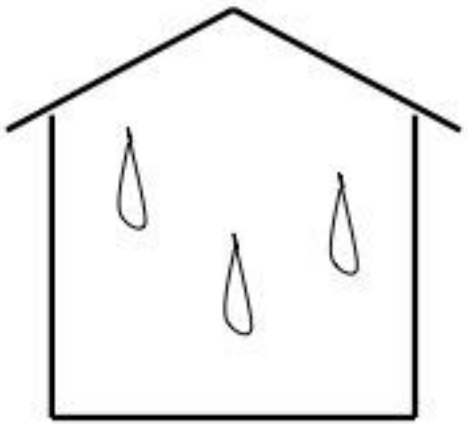
Scientific literature - surrogates of mold exposure used in population studies:

- 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

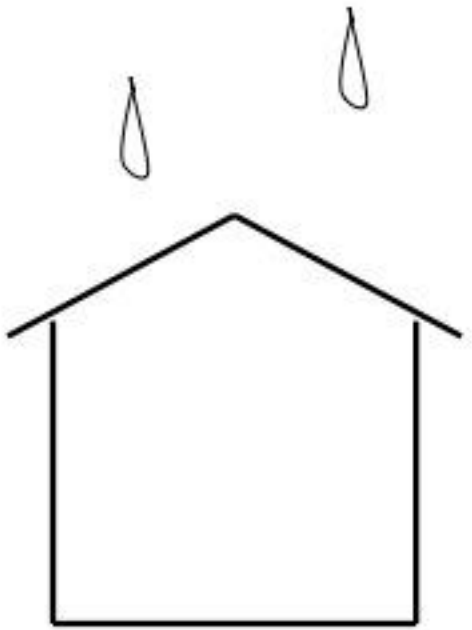
Indices of ”dampness” or ”mold” - a simplified summary

- 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!

Unhealthy



Healthy

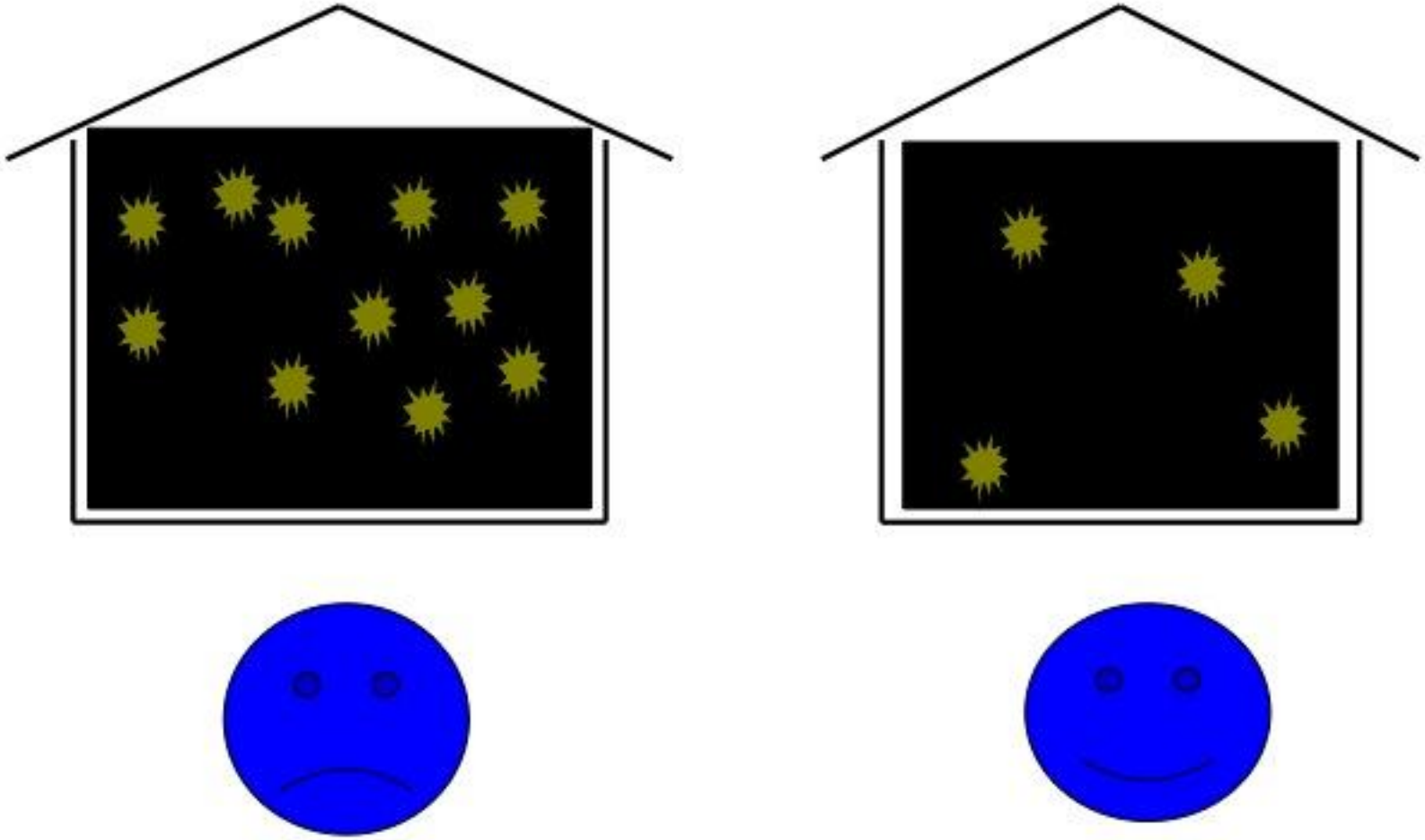


Individual cases of building mold are complex and diverse

- "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?



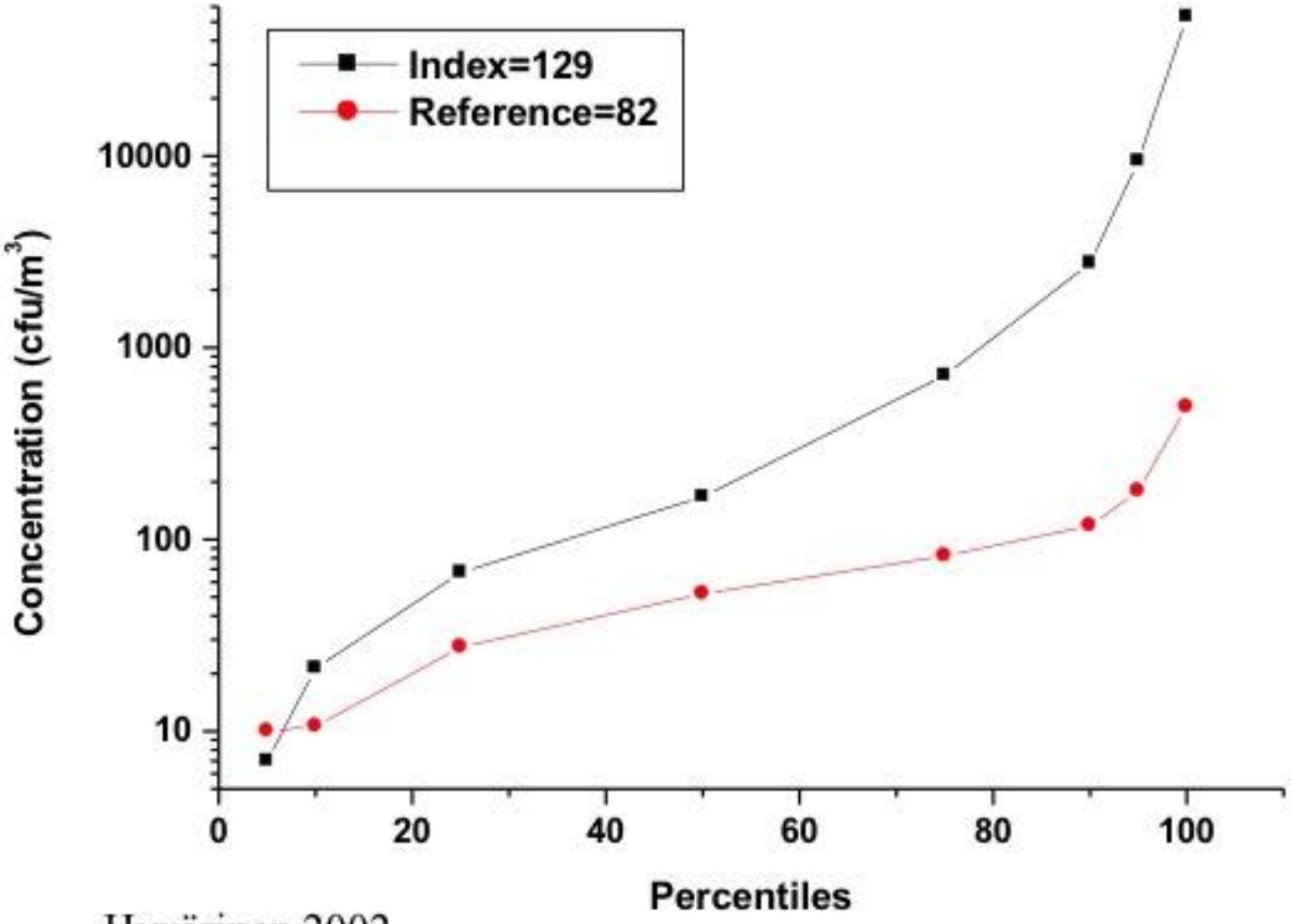
Scientific evidence between airborne microbial concentrations and health is weak

- 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)

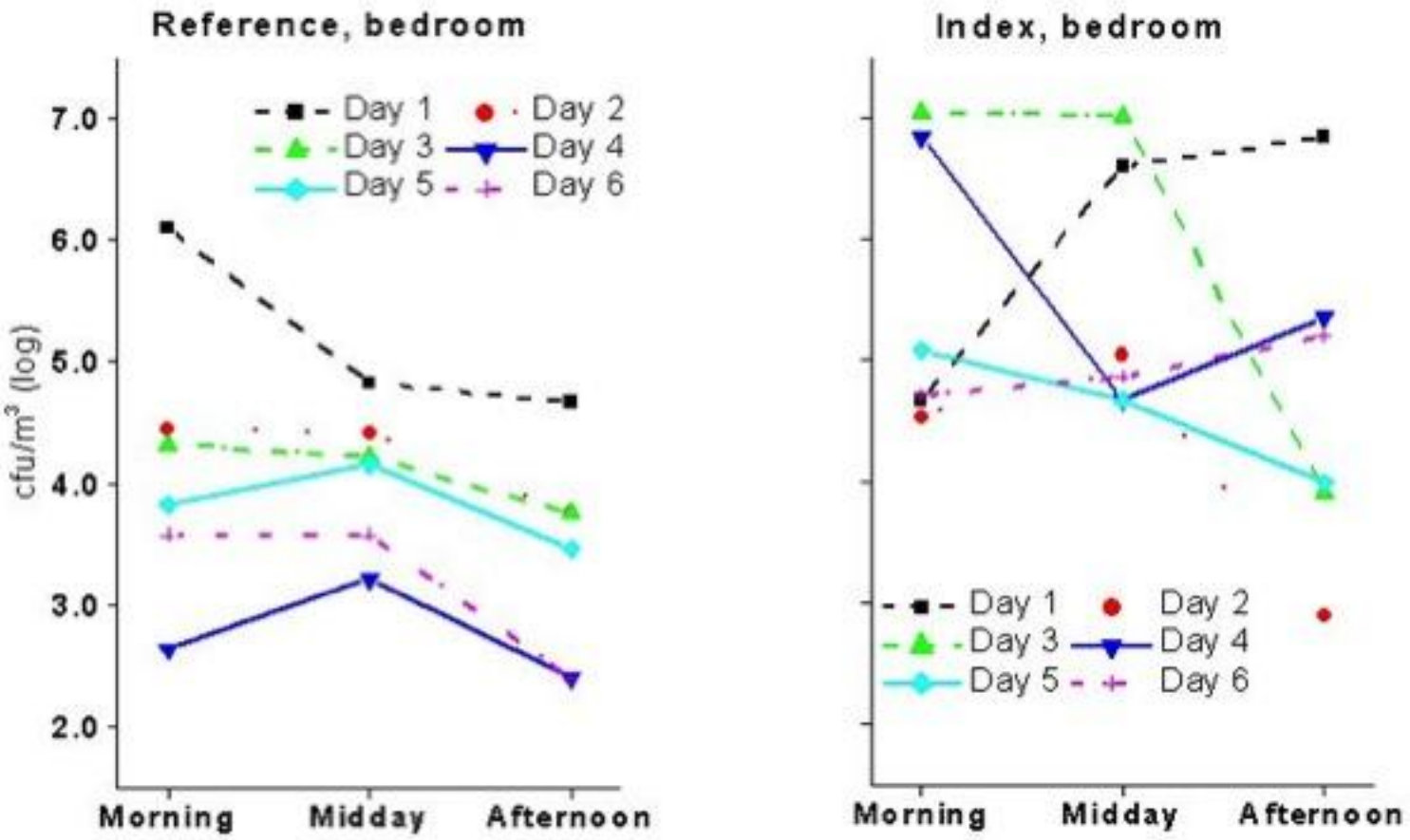
Some estimates of microbial concentrations indoors

- Concentrations <100 cfu/m^3 often considered "low",
- >1000 cfu/m^3 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

- *Acremonium*
- *Alternaria*
- *Aspergillus*
- *Aureobasidium*
- *Botrytis*
- *Chaetomium*
- *Cladosporium*
- *Doratomyces*
- *Eurotium*
- *Fusarium*
- *Geomyces*
- *Gliocladium*
- *Humicola*
- *Mucor*
- *Oidiodendron*
- *Paecilomyces*
- *Penicillium*
- *Phialophora*
- *Phoma*
- *Rhinocladiella*
- *Rhizopus*
- *Rhodotorula*
- *Scopulariopsis*
- *Sphaeropsidales*
- *Stachybotrys*
- *Torula*
- *Trichoderma*
- *Tritirachium*
- *Ulocladium*
- *Verticillium*
- *Wallemia*
- Yeasts

Examples of bacterial genera found in moldy building materials

- *Acinetobacter*
- *Agrobacterium*
- *Artrobacter*
- *Bacillus*
- *Brevibacterium*
- *Cellulomonas*
- *Clavibacter*
- *Corynebacterium*
- *Dietzia*
- *Flavobacterium*
- *Gordonia*
- *Methylobacterium*
- *Microbacterium*
- *Mycobacterium*
- *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

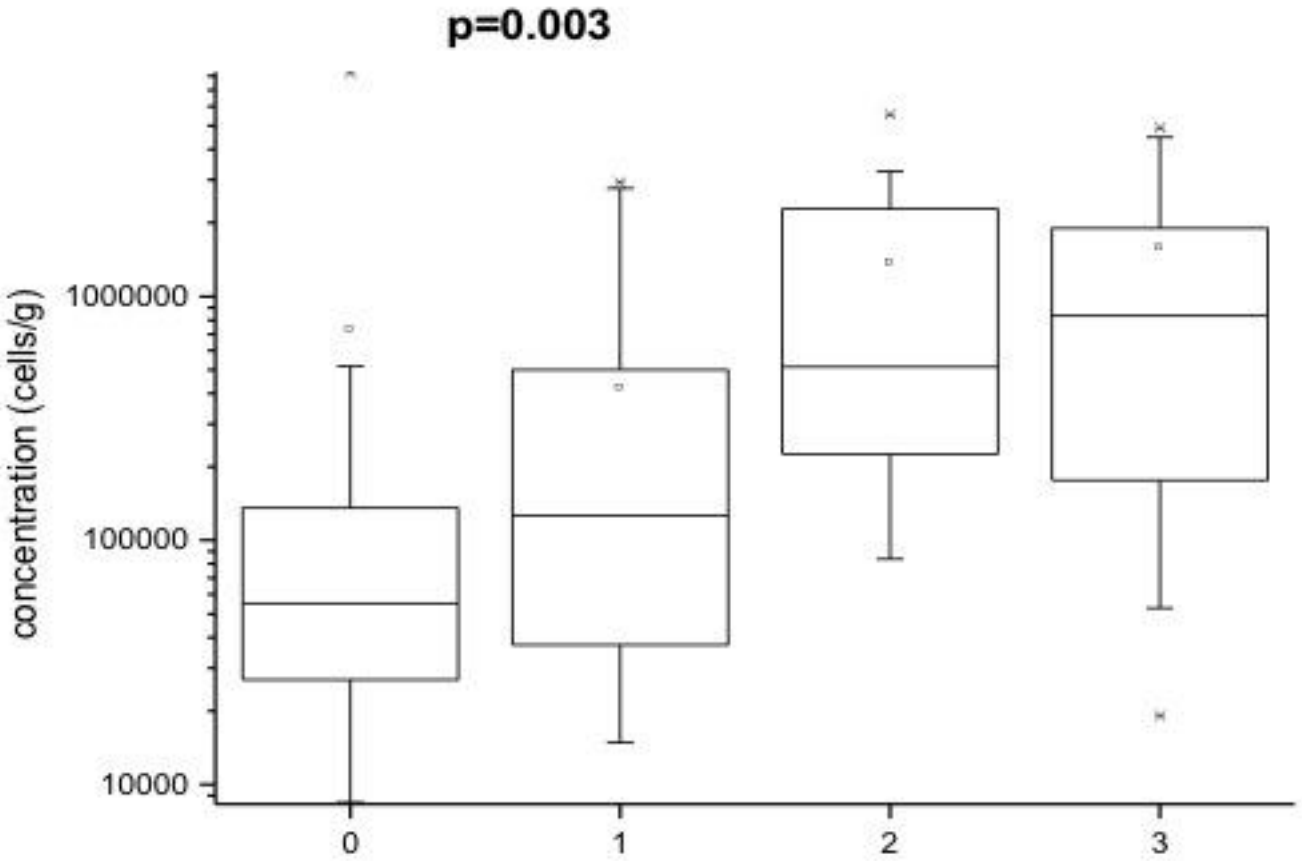
Other methods to assess microbial exposures

- 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

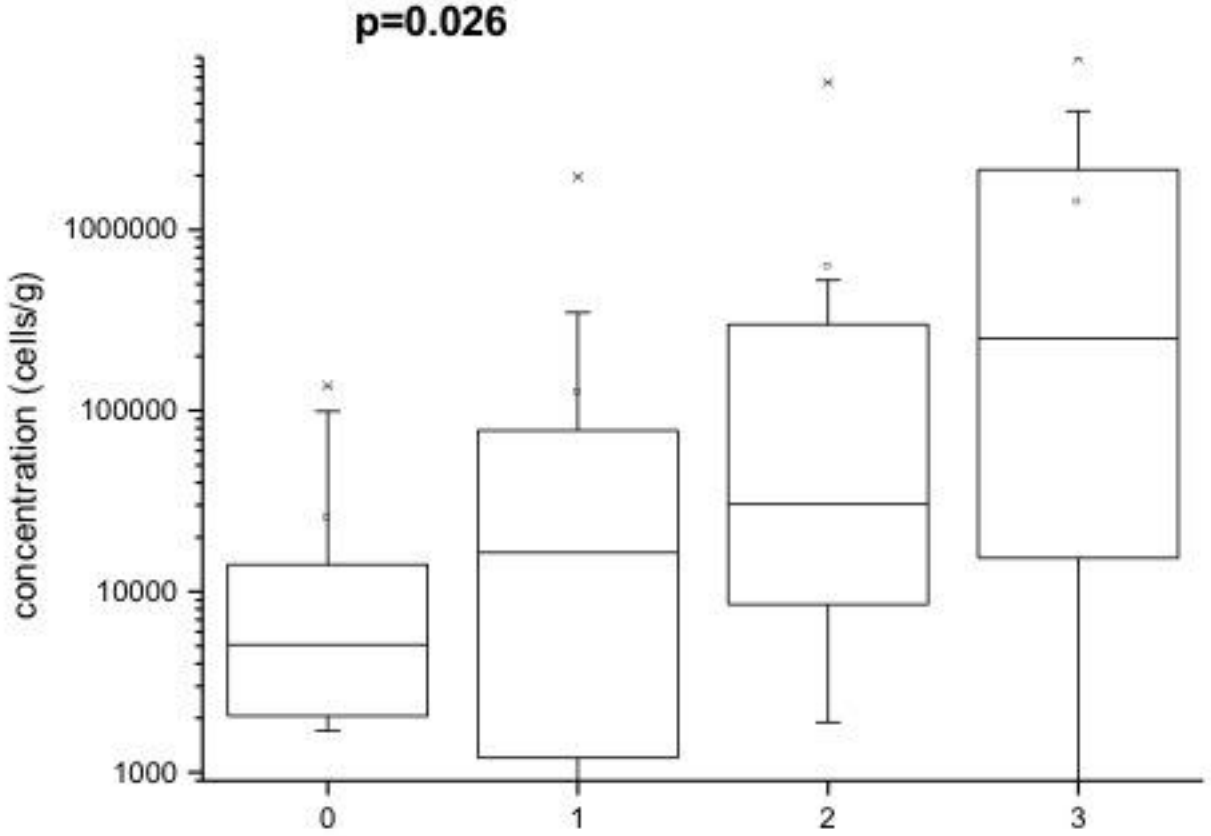
- Most abundant fungi in house dust
  - *Asp/Pen/Paec* (median 5.44×10^6 cells/g)
  - *Aureobasidium pullulans* (median 4.35×10^6 cells/g)
  - Concentrations $10^2 - 10^4$ times higher than culture results

Penicillium brevicompactum (QPCR) in house dust vs. moisture damage



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



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 class room increase concentrations of airborne bacteria – muramic acid as a bacterial marker (Fox et al.2005)

Mur in dust/pmol mg^{-1}		Mur in air pmol m^{-3}	
Unoccupied	Occupied	Unoccupied	Occupied
18.2 ± 9.6	115.5 ± 43.6	0.14 ± 0.10	6.97 ± 4.82
8.9 ± 4.4	109.2 ± 45.9	0.10 ± 0.04	3.47 ± 1.69
17.1 ± 14.3	80.2 ± 33.5	0.20 ± 0.28	4.71 ± 2.44

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)

	Difference
Building characteristics	
Moisture damage	yes
Visible mold	yes
Symptoms	
Respiratory symptoms	yes[1]
Respiratory infections	yes[1]

[1]*Husman et al. (1993), Koskinen et al. (1995)*

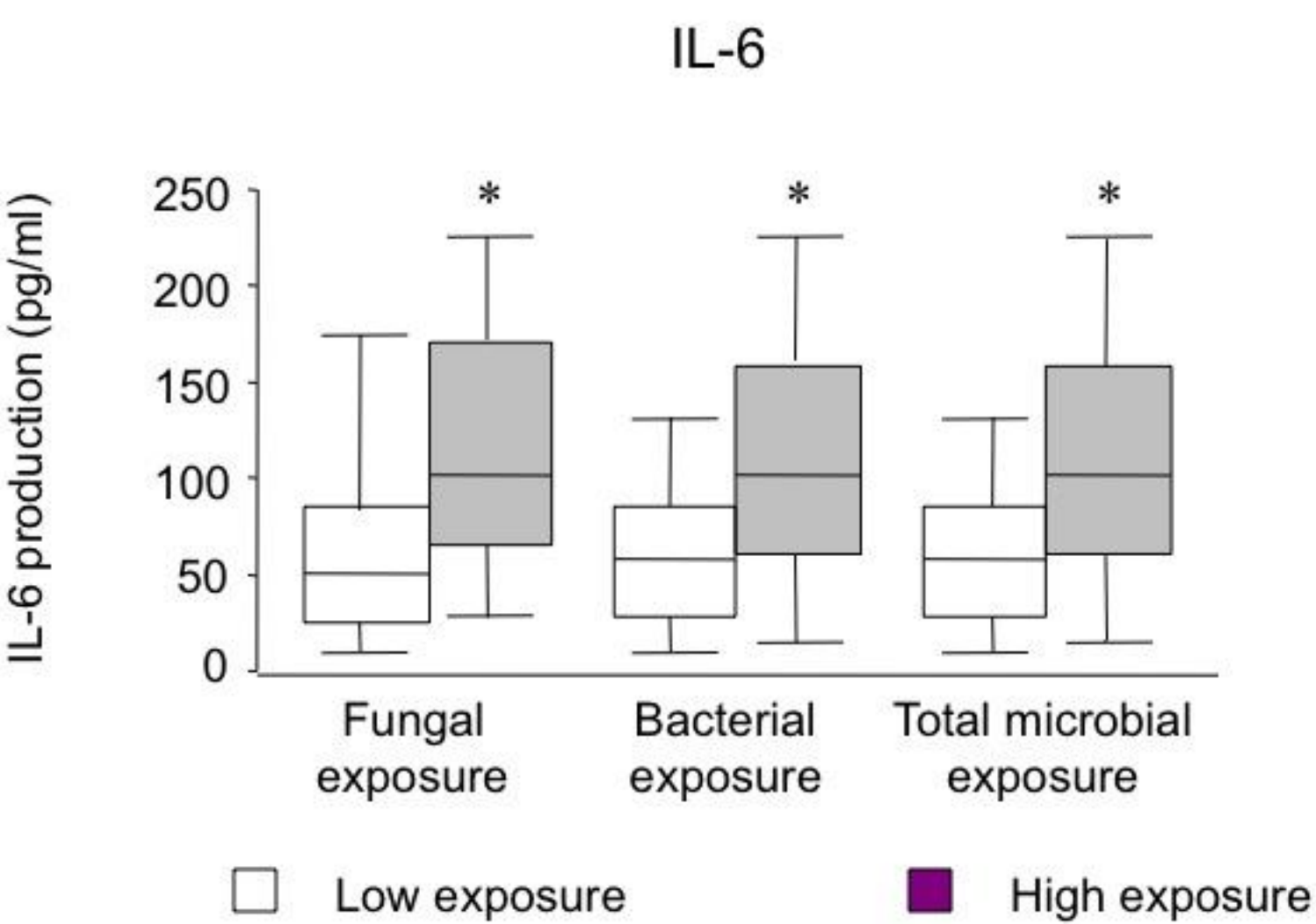
Summary of differences between index- and reference buildings

(Hyvärinen et al. 2001)

Parameters of indoor air quality	Difference
Airborne concentrations of viable fungi	Yes
Size distribution of viable fungi	Yes
Fungal composition of air	Yes
Airborne concentrations of viable bacteria	No
Concentrations of formaldehyde	No
Concentrations of TVOCs	Yes?
Concentrations in MVOC	No?
Indoor air temperature	Yes
Indoor air relative humidity	Yes

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...

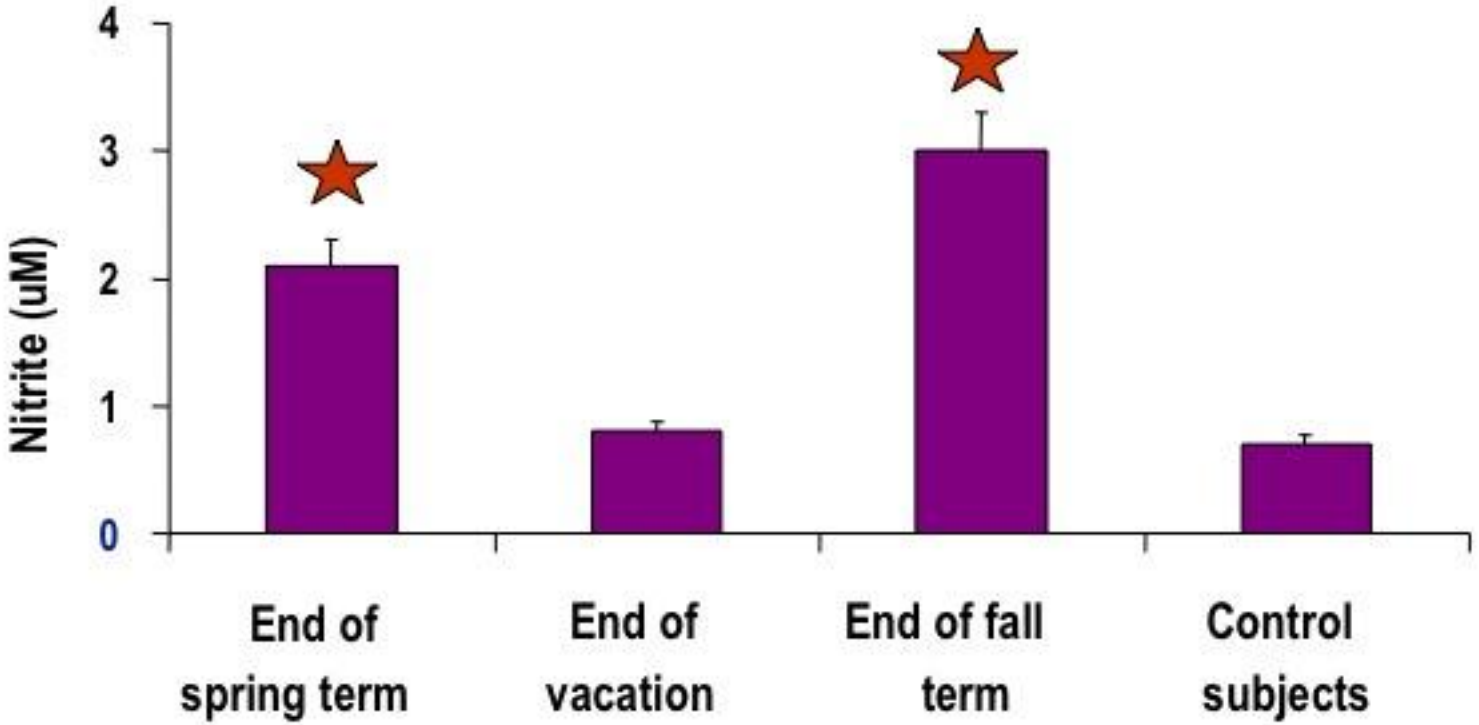


Biological activity of the particle material collected on filters in relation to low or high personal microbial exposure (Roponen et al., Inhal Toxicol 2003;15:23-38)

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
Am. J. Respir. Crit. Care Med. 1999; 160:1943-1946

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-*Pen/Asp* (Douwes et al. 2006), 1,3-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

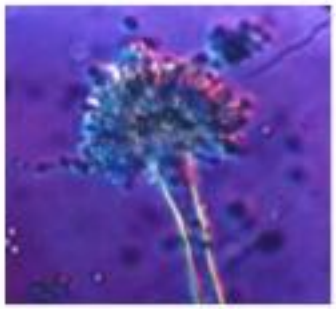
Conclusions

- 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