Microbial growth – a potentially harmful source of microbial agents

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Microbes and microbial products are everywhere

- Microbes are always present – where do they come from?
- Outdoor sources
- Indoor sources – normal and harmful
- It is obvious that some sources are more harmful than others
- What could be the explanation?
- Epi evidence on associations between mold growth and adverse health effects (not between microbial counts and health effects)

Sources of indoor fungi

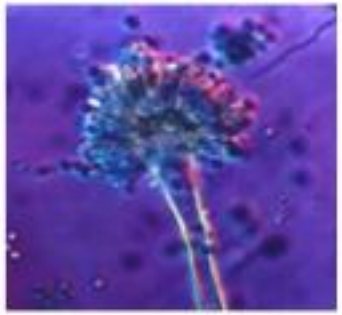
- Normal sources
  - Outdoor air
  - Foodstuff, firewood, pets' food, bedding
  - House dust (reservoir)
  - Users of the building; transport on peoples' clothes, on pets' fur
  - Colonies on wet surfaces
    - bath, kitchen, toilet, sinks, plumbing, plants, food, fruit, vegetables, any organic material

Sources of indoor fungi

- Non-normal source
  - Microbial growth on damp or wet surfaces
  - one source among others, but has more health relevance than others

Microbes in indoor air – normal situation

- Act as any particles while airborne
  - Depending on particle size, density
- Microbial material may occur in size range 10 nm-100 µm
- Continuous traffic from outdoors, occupants and indoor sources
- Settling on floor and surfaces, resuspension
- Removal by cleaning and ventilation



Normal microbes - friendly companions?

- Normal flora of skin is necessary for health
  - become air pollutants when released to air
- ”normal” mycobiota of indoor air similar as outdoors
- Possible protection from allergy
  - Microbes of farming environment

Harmful pollution?

- Infectious pathogens
  - source: humans
  - Alertness a necessary policy
  - Role of ventilation
  - Importance of cleaning
- Mold growth on moistened surfaces
  - Risk for many adverse health effects
  - Toxin production a potential risk
  - Renovation and clean-up a necessary policy

It matters where they come from

- Microbes are present everywhere;
- People have symptoms in certain indoor environments but get better when elsewhere
- In the context in question, no one gets sick from outdoor microbes, or microbes from normal sources
- It is the dampness, moisture and consequent mold growth that is the critical issue

A harmful source should be eliminated

- In case of building mold, is not enough to ventilate airborne microbial agents away,
- one should eliminate the source

- Learning more about the harmfulness of micobial growth:
  - It is the source we should focus on: what grows there, what are the metabolites produced

Indoor environments as habitats of microbes…

- Typically dry habitats compared to most natural environments;
- Nutrition available in buildings
  - Biomaterial from humans, animals, plants, house dust, nutrients of tapwater, sewage water…
- No extreme temperatures – not too cold, not too warm
- No extreme pH
  - except concrete; high pH limits the diversity of microbes growing on it

When does harmful growth start?

- All the other necessary factors for growth are there… just add the water
- Excess water may come from
  - condensation, leakage, rain, snow, flooding, capillary raise of soil water…
- Spores present everywhere, those microbes start to grow for which the niche is most optimal
- Growth will start within hours or days
- The longer the growth may continue – often months or years – the more diverse the growth becomes and the more potential for toxin production

General rules of microbial growth

- Wherever and whenever excess moisture, some microbial growth will take place
- The more water, the more mold
- Moisture sometimes fluctuating; microbial growth follows the fluctuation
- Drying the substrate (and mycelium) does not destroy the microbes; spores are there to wait conditions ”improve”

What will grow there?

- Microbial growth involves various species
- Never a pure growth of a single fungus
- Communities of species, an ecosystem develops
- Usually start with molds, yeasts and bacteria
- Later amoebae and nematodes, ants, insects…
- Microbial interactions are important for toxin production

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

Actinomycetes and other actinobacteria

- Actinomycetes (mainly *Streptomyces* spp.) occur commonly in mouldy materials
- Produce earthy odour, ”potato cellar” odour
- Most commonly (48%) on ceramic materials (Hyvärinen et al.2002)
  - Occur together with *A.versicolor, Acremonium*
- Mycobacteria in 23% of samples (Torvinen et al. AEM 2006;72:6822-6824)
  - Occur together with actinomycetes, *Aspergillus* spp., *Fusarium* spp. and yeasts

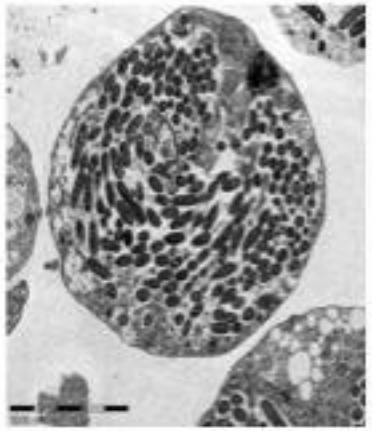
Indoor environments are very diverse in their microbial communities

- 200-300 fungal genera cultured
- With sequence analyses of the fungal communities, 5-10 times more genera found than with culturing
- Indoor bacteria less characterized so far
  - Analyses show a rich diversity, too
  - Dominated by gram positive species

Microbial growth on a building material may also include amoebae

(Yli-Pirilä et al. 2004, 2006, 2007, 2009)

- Amoebae occurred in appr. 20% of samples of moldy materials
- Amoebae may protect bacteria growing inside them: *Chlamydia*, mycobacteria etc.
- Presence of amoebae influenced the growth and toxicity of other microbes

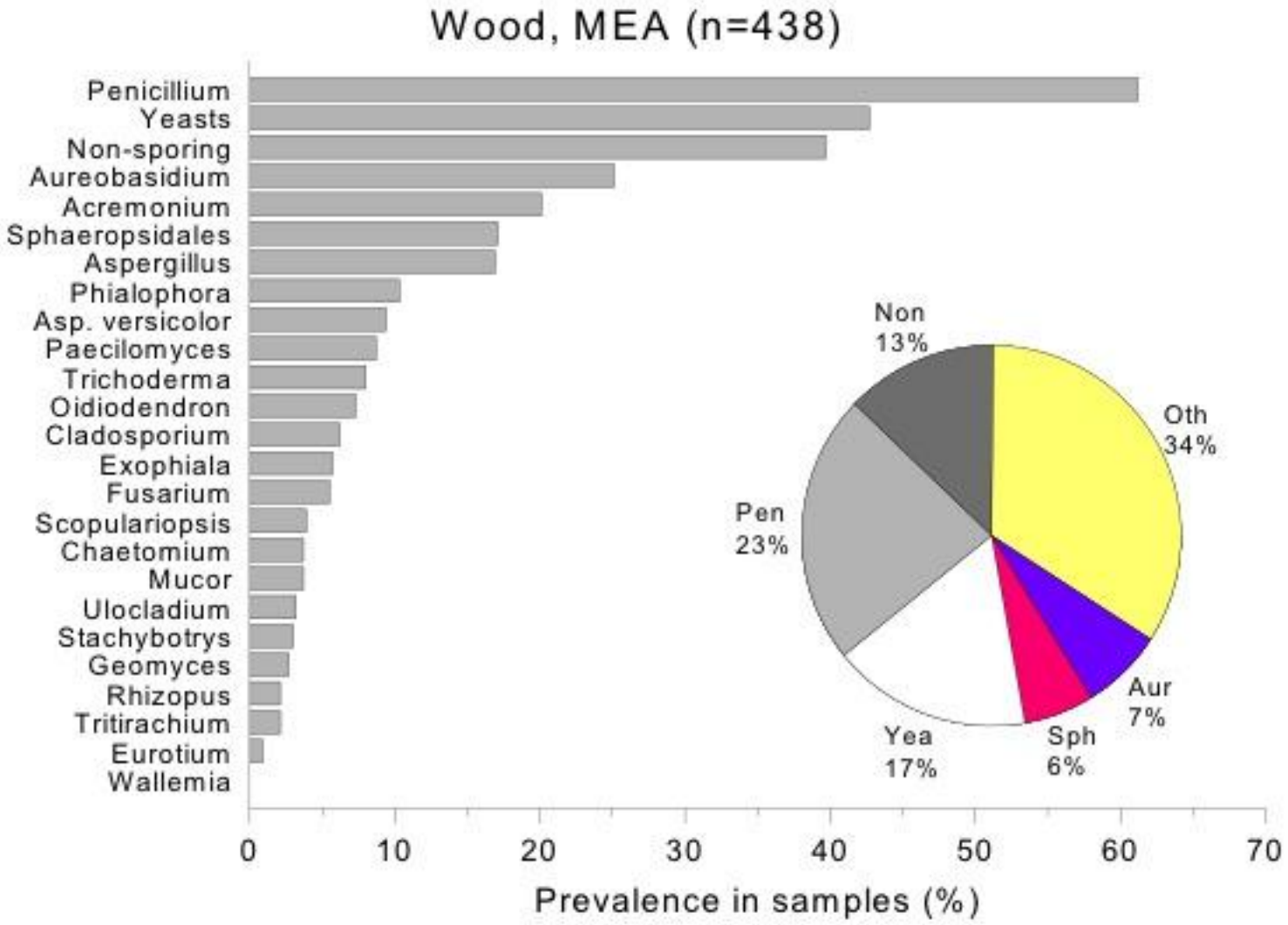


Role of material on microbial growth

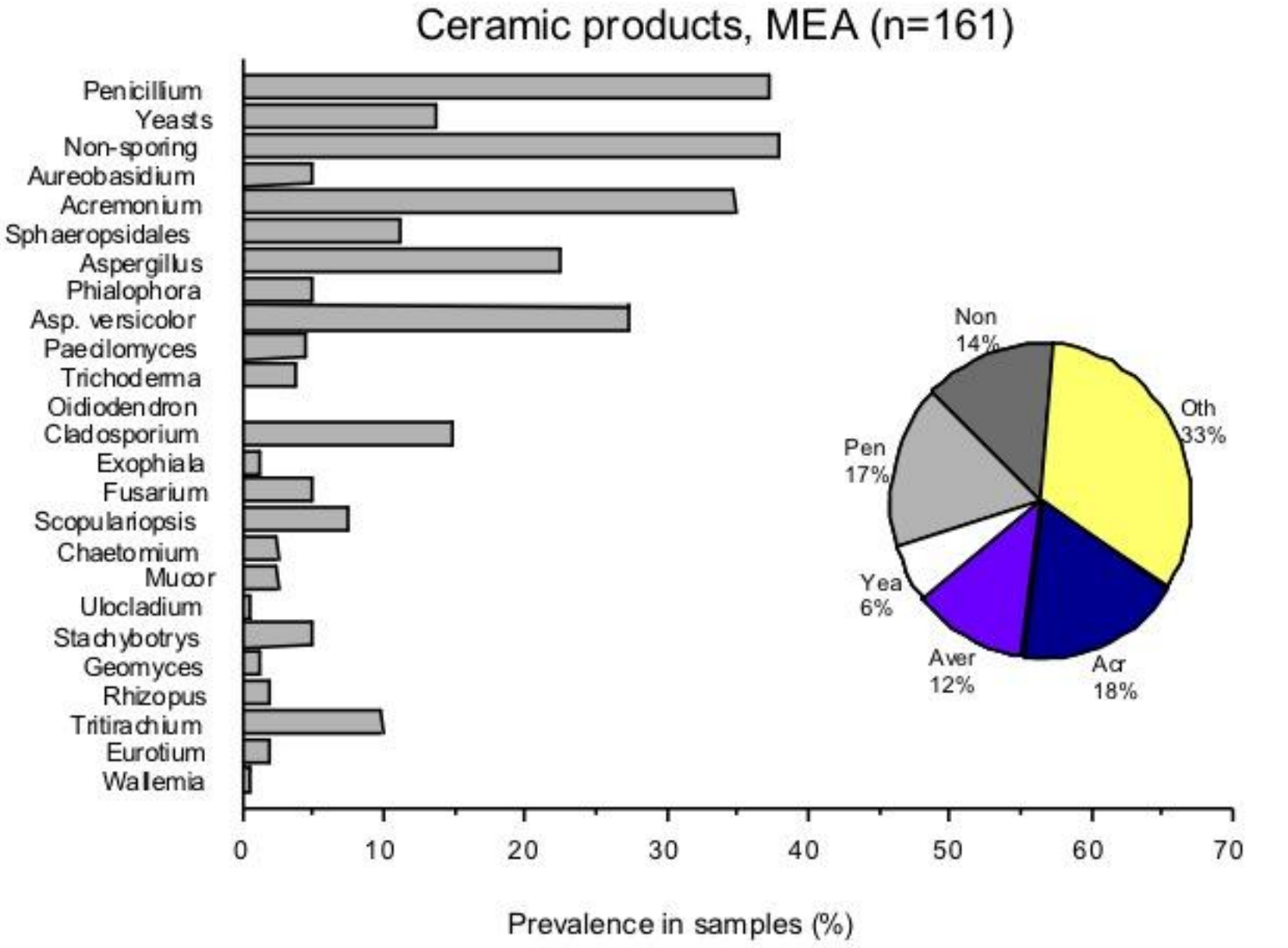
- Type and content of substrate is one factor regulating microbial growth
- Substrate has also a role in regulating secondary metabolism, e.g. toxin production

Study on the distributions of fungi in various building materials (Hyvärinen et al. 2002)

- Fungi were analyzed from damaged samples
- Quantitative counts and qualitative analyses on genus level
- Statistical analyses on occurrence of fungi on certain materials
- Materials differed for their fungal content



Hyvärinen *et al.* 2002



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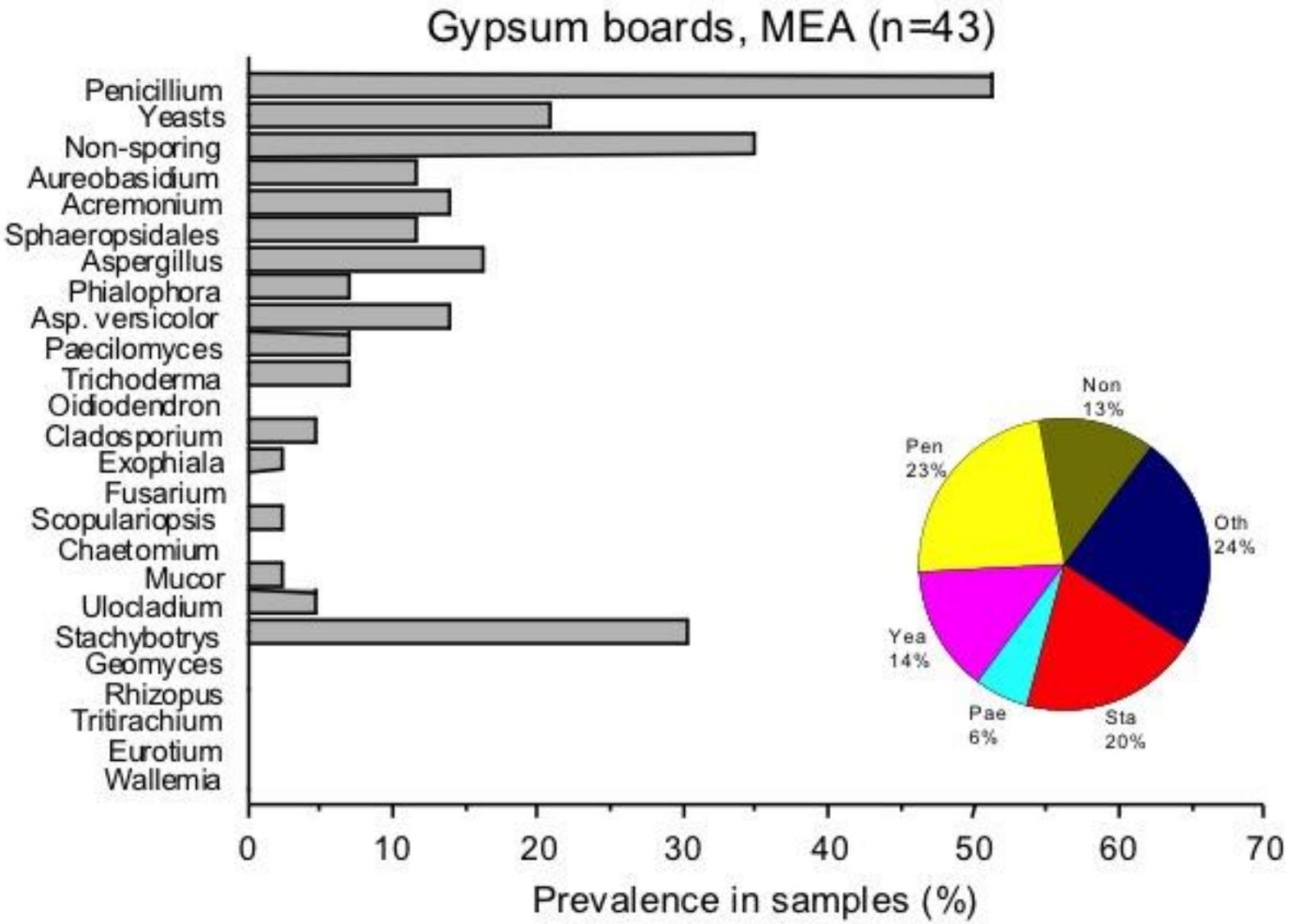

Hyvärinen *et al.* 2002

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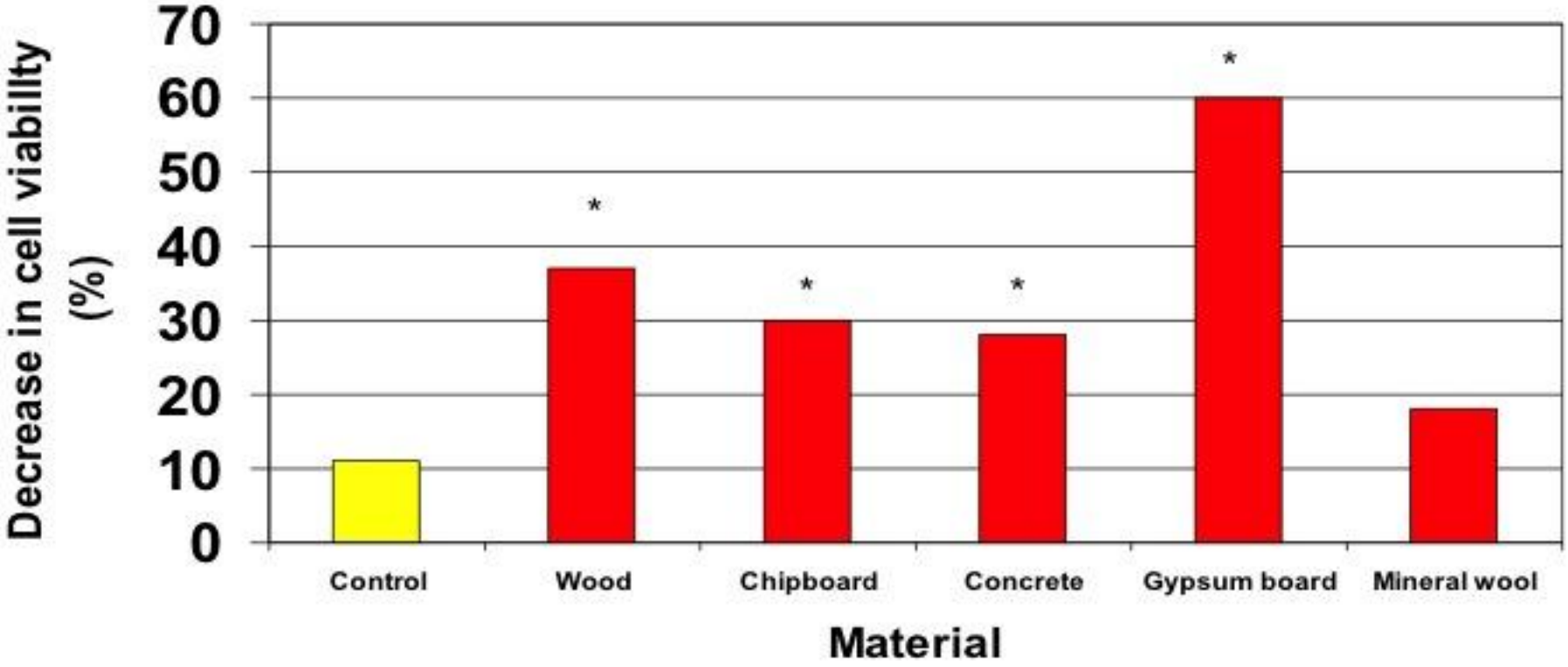
Substrate/material also affects the toxicity of microbial growth

- The same streptomycete strain *(S.anulatus)* was cultured on wetted pieces of building materials in small chambers for 2 months
- Spores were collected to a suspension which was used to expose cell culture
- Cytotoxicity was assessed as percentage of dead cells from total (MTT test)
- *S. anulatus* was most toxic after growth on gypsum board

Importance of material to microbe's toxicity



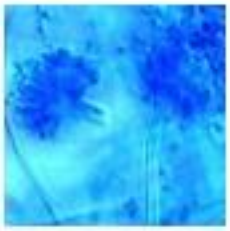
Cytotoxicity induced by *Streptomyces anulatus* grown on different building materials. Roponen *et al.* Indoor Air 2001;11:179-184

Occurence of toxic bacterial and fungal metabolites on mold damaged building materials

Microbial metabolites (positive/total samples)	Detection of metabolites in 9 building material samples								
	4105	4106	4107	4108	4109	4110	4111	4112	4113
Fungal									
Chaetoglobosin A (8/9)	+	+	+	+		+	+	+	+
Emodin (5/9)	+	+		+		+			+
Meleagrin (9/9)	+	+	+	+	+	+	+	+	+
Roquefortine C (3/9)			+				+	+	
Stachybotrylactam (4/9)			+			+		+	+
Sterigmatocystin (6/9)	+	+		+	+			+	+
Trichodermol (1/9)								+	
Bacterial									
Monactin (3/9)					+	+		+	
Valinomycin (6/9)		+		+	+	+		+	+
Number of different metabolites per sample:	**4**	**5**	**4**	**5**	**4**	**6**	**3**	**8**	**6**

Intervention studies

- Intervention: **removal of the source** of microbial contamination
- may involve improvement of ventilation other renovation
- effects of the elimination of source
  - on building condition
  - on IAQ
  - on occupant health



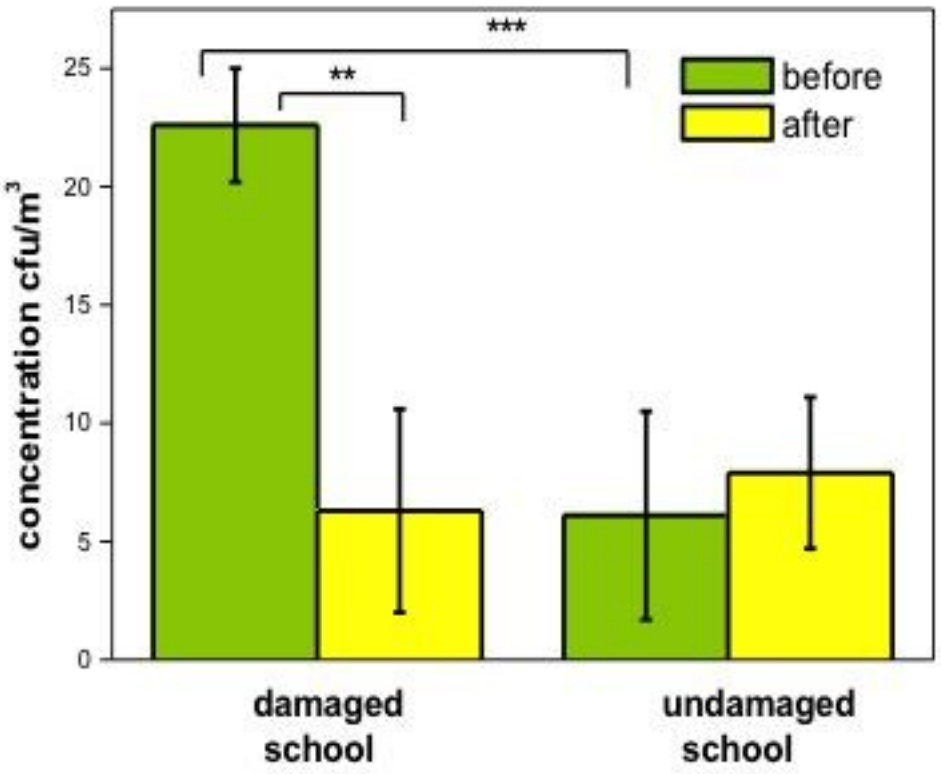
Remediation of two school buildigns

- 2 problem schools, one completely renovated, one partly renovated
- 2 control schools
- microbial measurements and symptom questionnaire before and after remediation

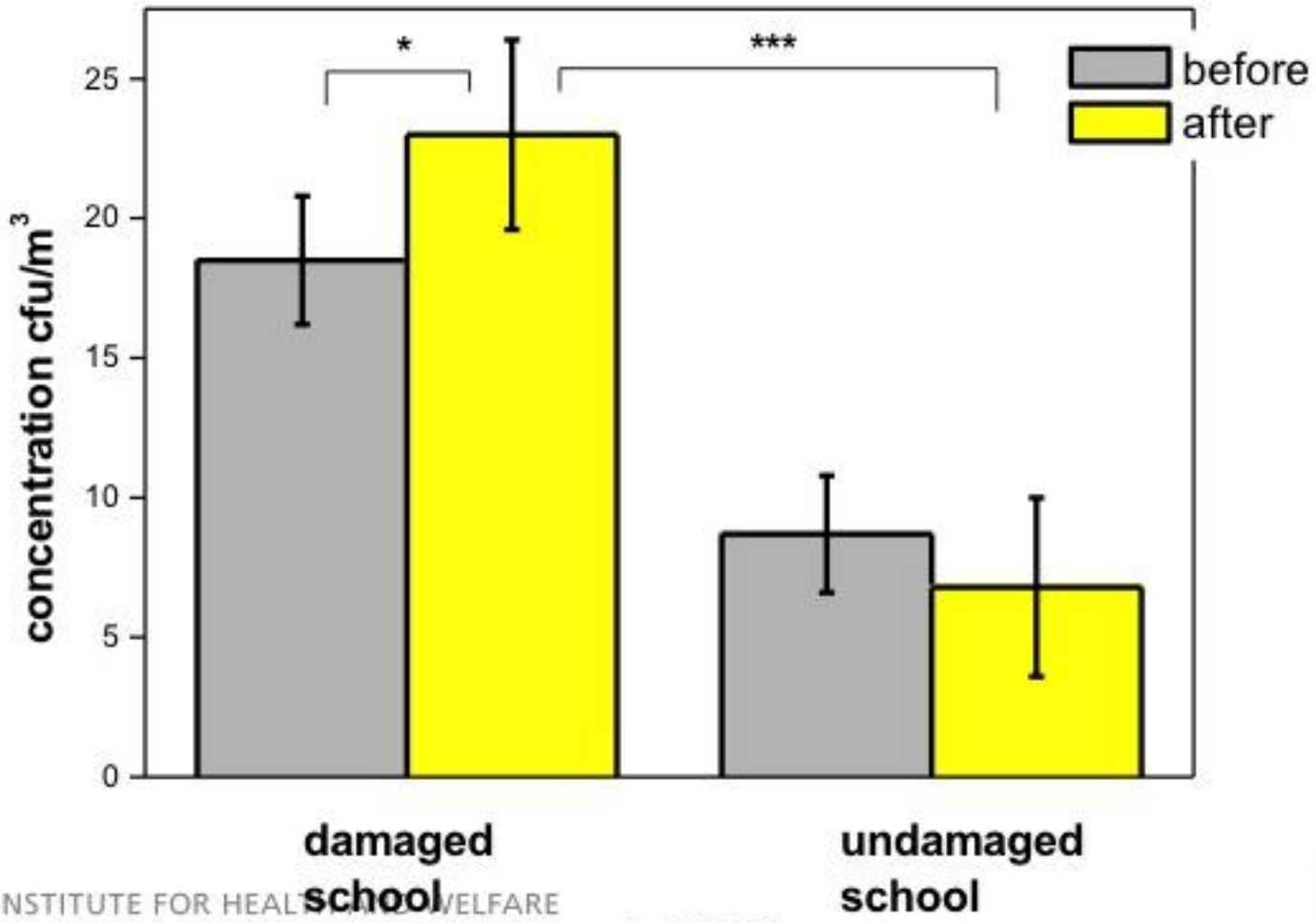
Meklin et al. (2005)

Airborne fungi before and after school remediation

- Concentrations of airborne fungi decreased to normal
- Note: airborne concentrations low even before remediation;
- Difference significant

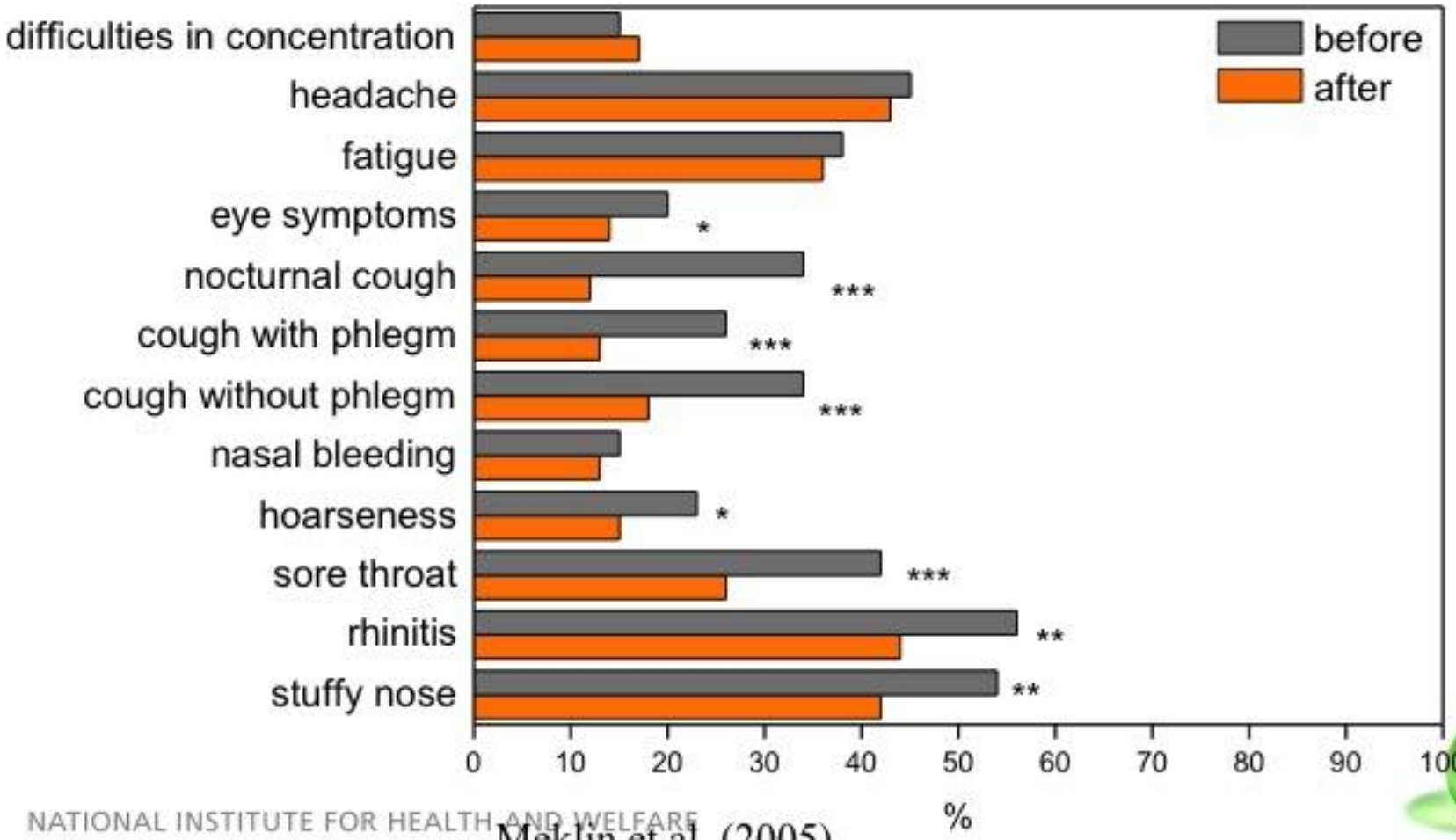


Fungal concentrations / partial remediation



Meklin et al. (2005)

Symptom prevalence / complete remediation



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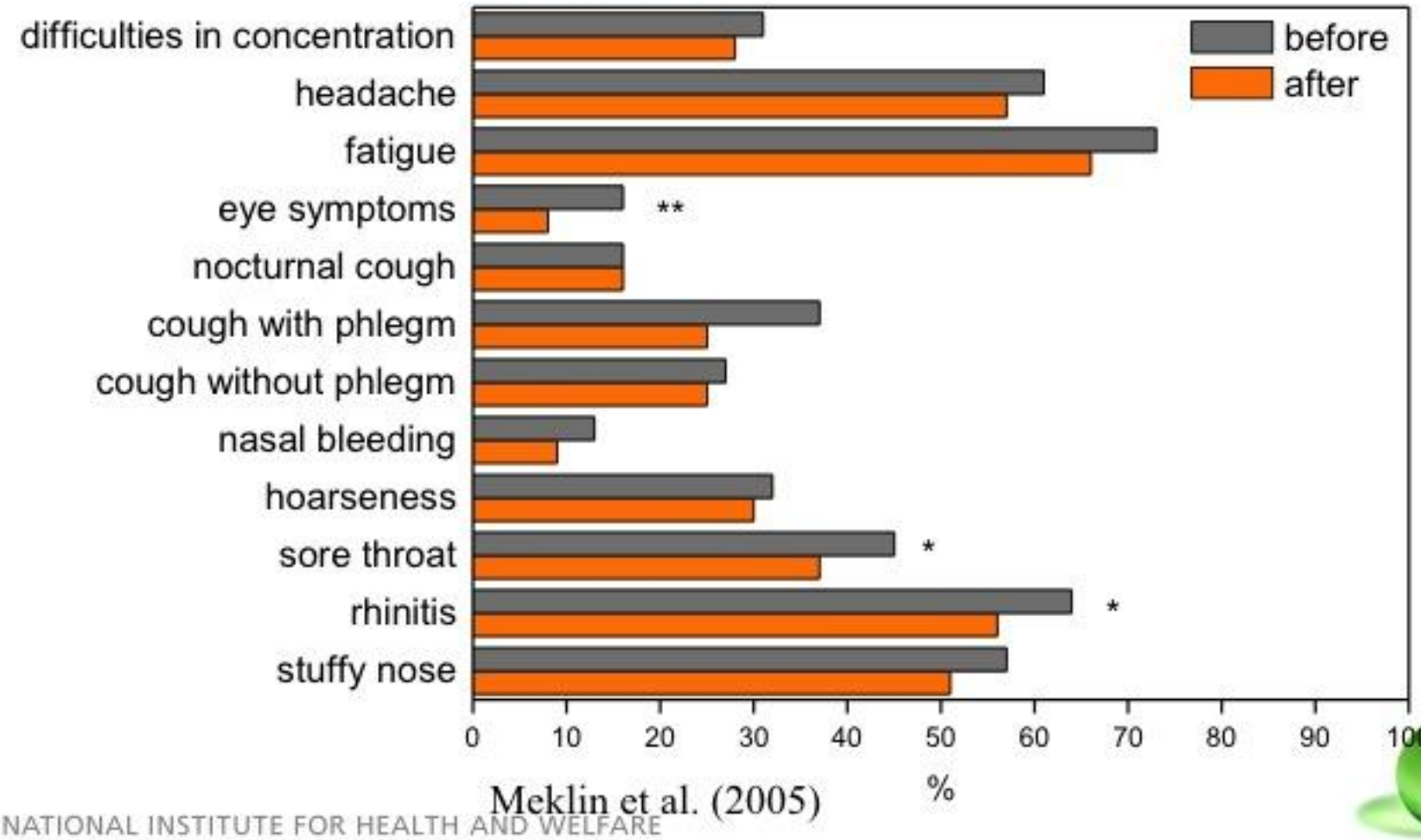
Symptom prevalence / partial remediation



Meklin et al. (2005)

Conclusions from the school intervention study

- Fungal concentrations were a marker of harmful exposure; higher than control before renovation
- Decreased to ”normal” levels after a thorough remediation, elimination of the source(s)
- Many symptoms decreased as a result of remediation
- After a partial remediation, fungal concentrations higher than before
- Some symptoms decreased; was their cause eliminated?

School study II
(Haverinen et al. 2004)

- *The results indicated that the repairs succeeded in the sense that new cases of symptomatic students were no longer identified*
- Reversibility of the symptoms of exposed individuals?[1]
- Time period needed for symptom relief?

- [1]See also Rudblad et al. Nasal histamine reactivity among adolescents in a remediated moisture-damaged school – a longitudinal study. Indoor Air, 14, 342-350 (2004)

… Effects of moisture remediation on health of adults. Summary of reported intervention studies. (Patovirta 2005)

	Place/ Number of participants	Methods	Health effects of remediation
Rudblad et al 2002	school n=28	questionnaire nasal provocation test	increased reactivity to histamine in nasal provocation test
Ebbehoj et al 2002	swimming bath n=25	questionnaire clinical examination 2-week peak flow follow-up	decrease in symptom levels decrease in peak-flow variability
Patovirta et al 2003	school n=26	questionnaire IgG-antibodies	the connection between elevated IgG-antibodies and sinusitis
Patovirta et al 2004	school n=44	questionnaire spirometry	no new asthma cases respiratory infections decreased
Patovirta et al 2004	school n=56	questionnaire	symptoms of fatigue and headache decreased

Effects of moisture remediation on health of adults. Summary of reported intervention studies. (Patovirta 2005)

	Place/ Number of participants	**Methods**	**Health effects of remediation**
Jarvis and Morey 2001	11-story structure n=488	questionnaire	lower symptom rates no new building related respiratory disease
Åhman et al 2000	school n=21 teachers n=224 pupils	interview	lower almost "normal" symptom rates
Sigsgaard et al 2000	school n=43	questionnaire	mucosal and neurological symptoms decreased
Sudakin 1998	office building n=37	interview	decrease in upper respiratory and neurobehavioral symptoms

Growing microbes - bad microbes

- Microbial growth is the source of harmful agents in damp or moisture damaged building
- microbial growth in the building acts as a source of particle and volatile emissions into indoor air
- It is important to eliminate the source, not to paint on it or encapsulate it