Microbial Exposure and Health in Schools –
Effects of Moisture Damage and Renovation

Teija Meklin

National Public Health Institute
Department of Environmental Health
P.O.Box 95, FIN-70701 Kuopio, Finland

and

University of Kuopio
Department of Environmental Sciences
P.O.Box 1627, FIN-70211 Kuopio, Finland

ACADEMIC DISSERTATION

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Mannerheimintie 166
FIN-00300 Helsinki, Finland
phone +358 9 47441
telefax +358 9 4744 408

Author’s address: National Public Health Institute
Department of Environmental Health
P.O.Box 95, 70701 Kuopio, Finland
phone +358 17 201364
telefax +358 17 201155
email teija.meklin@ktl.fi

Supervisors: Dosent Aino Nevalainen, Ph.D.
National Public Health Institute,
Kuopio, Finland
Professor Pentti Kalliokoski, Ph.D.
Department of Environmental Sciences
University of Kuopio, Finland

Reviewers: Dr. Markku Seuri, M.D.
Kuopio Regional Institute of Occupational Health
Kuopio, Finland

Dosent Antti Tossavainen, Ph.D. (technol).
Uusimaa Regional Institute of Occupational Health
Helsinki, Finland

Opponent: Dosent Antti Koivikko, M.D.
Department of Paediatrics
University of Turku
Turku, Finland

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ABSTRACT

A total of 32 school buildings were studied to determine whether the microbial indoor air quality and associated respiratory symptoms among children in schools with visible moisture and mold problems differed from those in non-damaged schools. Also, the effect of the building frame (concrete/brick or wood) of schools was analyzed and the size distributions of airborne microorganisms in school buildings were considered. A total of 5345 children returned the symptom questionnaire.

To study the effects of moisture and mold damage repairs on microbial exposure and symptom prevalence in the schools, four school buildings were selected to the study. Samplings of indoor air microbes were performed identically before and after repair works in the damaged schools. Change in symptom prevalence caused by repairs was studied before and after repairs in the cross-sectional surveys. Comparable surveys were done in two non-damaged schools. Over 1300 schoolchildren participated the study.

The type of building frame material affected the microbial content of the building; mean concentrations of fungi were significantly higher in the school buildings of wooden construction than in the schools with a concrete/brick frame. An association between concentrations of fungi and moisture damage was found in concrete schools, but not in wooden schools. Typically, in moisture-damaged school buildings of concrete construction, the geometric mean wintertime concentration was above 10 cfu/m³, there was a low frequency of samples with values under the detection limit, and the frequent occurrence of samples with concentrations above 50 cfu/m³.

Elevated concentrations of Cladosporium and actinobacteria (concrete schools) and the occurrence of Aspergillus versicolor, Stachybotrys and Acremonium (both frame types of schools) were associated with moisture damage. The average geometric mean diameter of total viable fungi was smaller in the wooden schools than in the concrete schools, and smaller in the moisture-damaged than in the reference schools.

Moisture damage in the school building was a risk factor for respiratory symptoms among schoolchildren. The association between moisture damage and respiratory symptoms was statistically significant only in the concrete schools. Indoor characteristics causing discomfort were also more often reported in the damaged schools than in the reference schools.

After a thorough renovation of moisture- and mold damage in a school, the levels of airborne microbes and the fungal diversity of the samples normalized to the level in the reference school. Also, a remarkable decrease in prevalence of 10 symptoms out of studied 12 symptoms among schoolchildren was achieved. After only partial repairs, an increase of contamination was detected in the air samples. An improvement in symptom prevalence was less marked than after thorough renovation.
To Riku, Reetta, Juhani and Elina
To my parents
Olavi Pelkonen
and late Leena Pelkonen
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Teija Meklin
ABBREVIATIONS

ac/h  air change per hour
aw  water activity
CFU  colony forming units
d_{g,ave}  average mean diameter
DG18  dichloran 18% glycerol agar
DL  detection limit
DNA  deoxyribonucleic acid
FEV\textsubscript{1}  forced expiratory volume in 1s
FVC  forced vital capacity
GM  geometric mean
HVAC  heating, ventilation and air conditioning
IAQ  indoor air quality
IgG  immunoglobulin G
MEA  malt extract agar
PVC  polyvinyl chloride
RCS  Reuter centrifugal sampler
spp.  species
TGY  tryptone glucose yeast agar
VOC  volatile organic compounds
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1 INTRODUCTION

In Finland, 600 000 children attended primary and secondary schools in 2001 and they were being taught by 42 000 teachers (Statistics Finland 1999, 2001). In a middle-sized Finnish town, on an average, 20.2 children are seated in each classroom in primary schools (School office, Kuopio). Due to the large number of people occupying school buildings, indoor air quality (IAQ) of schools should be recognized as a priority topic for public health.

High occupant density in schools is also an aspect highlighting the importance of good indoor air quality and adequate ventilation. As many as 40% of Finnish school buildings suffer from insufficient ventilation (Kurnitski et al. 1996). Poor ventilation leads to the accumulation of pollutants from different sources and may increase the incidence of symptoms among building occupants (Seppänen et al. 1999). Also, with regard to infectious diseases, the importance of good ventilation is obvious. For example, it has been shown that massive spread of measles by airborne transmission occurred in a school building with poorly ventilated hallway even though the students were vaccinated (Paunio et al. 1998). Poor ventilation may also indirectly contribute to moisture damage in a building by increasing the risk of condensation of water (Lstiburek and Carmody 1994). On the other hand, when ventilation is adequate and there is no moisture damage in buildings, then the risk of indoor air quality related diseases remains low (Sundell 2000), since effective ventilation dilutes all potential pollutants in indoor air.

There are various sources of pollutants in school buildings. Air contaminants are derived from moisture and mold-damaged materials and old or deteriorating furnishings, cleaning materials, likewise as emissions from new furnishing. Also, activities such as experiments in science laboratories and handwork training areas can be occasional sources of pollutants (Thompson 1998, EFA 2001). The occupants of the building are important sources of human-derived pollutants.

Moisture and mold problems in buildings are among the major factors affecting the indoor air quality. The association between moisture damage in buildings, microbial
growth due to excess moisture and adverse health outcomes of the occupants has been convincingly demonstrated in many epidemiological studies (Waegemaekers et al. 1989, Dales et al. 1991, Brunekreef et al. 1992, Spengler et al. 1994,). The risk of respiratory symptoms, such as cough and wheeze or asthma as well as respiratory infections and general symptoms like headache and tiredness, is higher for occupants in moisture damaged buildings (Peat et al. 1998, Bornehag et al. 2001).

The headmasters of Finnish schools have estimated in the questionnaire that moisture damage was present in 53% and serious damage as indicated by visible mold growth or mold odor in 26% of the school buildings (Kurnitski et al. 1996). Moisture damage repairs had been undertaken in about 30% of school buildings in Finland during the years 1996-1999. Unfortunately, these repairs have often been postponed for many years due to financial restraints. Recent reviews in Finland have shown that the need for repairs due to moisture damage in school buildings may even increase in the future (The Association of Finnish Local and Regional Authorities 2000).

Due to the high prevalence of moisture and mold damage in schools, especially since it can impact on human health, tools to evaluate and characterize the microbial status of the building are needed. The present guidelines for microbial sampling and interpretation of the results, however, are mainly based on findings from residential environments. Schools differ from homes in many ways; size, activities and occupant density may alter the microbial status in schools. Also, information about the effects of moisture damage repairs on microbial indoor air quality and the health status of schoolchildren is lacking.
2 REVIEW OF THE LITERATURE

2.1 Moisture damage and microbial growth

In principle, properly designed, built and maintained buildings should be able to remain undamaged (Lstiburek and Carmody 1994). This ideal situation is not always experienced in practice. Microbial growth may occur in buildings if the growth requirements of environmental microorganisms are satisfied. In general terms, moisture is the critical factor. Thus, the whole issue of microbial contamination focuses around moisture damage in buildings. The greatest moisture and water load comes from outdoors. Water leaks due to defects in roofs, foundations and walls are common (Flannigan and Morey 1996, Nevalainen et al. 1998, Chelelgo et al. 2001). Structural faults may lead to moisture damage after moisture movement due to water flow, capillary suction, air movement or vapor diffusion (Lstiburek and Carmody 1994). On the other hand, modern lifestyles require abundant use of water inside the building, and therefore, the risk of moisture damage is also high (Oliver 1997). Housing characteristics, such as ventilation and heating facilities, age of construction as well as building materials, may associate with high levels of humidity in the indoor environment (Hyndman 1990, Verhoeff et al. 1992). This may lead to moisture condensation on cold interior surfaces (Lstiburek and Carmody 1994).

In many types of climates, outdoor humidity determines the relative humidity levels in the indoor air. If not properly vented, dampness problems may occur due to condensation. This is not common in countries with cold climates having a prolonged heating season. According to the study by Chelelgo et al. (2001), only 12% of the Finnish houses and 33% of the apartments had relative air humidity higher than 45%.

In addition to the microbial growth, chemical deterioration is often related to moisture damage in building materials, degradation of components in polyvinyl chloride (PVC) floor coatings or carpet glues as an example (Norbäck et al. 2000a). Accumulation of mineral salts within and on the surface of materials can occur after moisture damage, because the penetrating water may contain mineral salts or water can act as a solvent for the salts naturally present in most building materials. Accumulation of
mineral salts may lead to erosion, flaking, or even total deterioration of the building materials (Oliver 1997).

2.1.1 Basic requirements for microbial growth

Vegetation, soil and decomposing organic material are continuous sources of microbial spores and cells, and spores are always present in the outdoor air. The snow cover on the ground reduces the concentrations in winter. When entering the building, the spores tend to settle down on interior surfaces depending on their aerodynamic properties. The growth of these environmental microbes is regulated by the environmental conditions. The most important factor is the water activity ($a_w$) of the building material. Its optimum is 0.95-0.99 for the mesophilic molds (Gravesen et al. 1994). According to field and laboratory studies, the colonization of molds is found to follow a distinct progression on gradually moistening building materials, i.e., the primary colonizers come first ($a_w < 0.80$), followed by secondary ($a_w 0.80-0.90$) and tertiary colonizers ($a_w > 0.90$) (Grant et al. 1989). Microbial growth associated with fluctuating moisture conditions is a complex phenomenon which also depends on the material in question (Adan 1994, Viitanen and Bjurman 1995, Korpi et al. 1998, Pasanen et al. 2000). The basic preconditions for fungal growth on a material include a temperature minimum, for most fungi this is 2-5 °C (optimum 22-27°C for mesophilic fungi), and a pH minimum (optimum 5-6.5). Organic substances can function as a source of carbon and nitrogen. The inorganic nutrients include potassium, phosphorus, magnesium, and sulfur. Therefore, various building materials differ in their potential to provide nutrients for microbial growth. Once the fungi have colonized a material, they are able to synthesize the vitamins they need for themselves (Ingold and Hudson 1993).

When enough water is available in building materials, nutritional factors become crucial as growth-limiting factors as shown in a study where, at a similar moisture content, a ceiling tile containing cellulose supported the growth of fungi whereas inorganic ceiling tiles did not (Karunasena et al. 2000). By increasing the nutritional content of the substrate, the minimum $a_w$ required for growth decreases (Grant et al. 1989, Foarde et al. 1996). However, germination of fungi also depends on temperature (Vujanovic et al. 2001). Nutritional conditions may also affect the toxic
properties of microbes, i.e. the same microbes can exhibit different biological responses when grown on different materials (Roponen et al. 2001, Murtoniemi et al. 2001). Microorganisms rarely exist alone but as mixed populations. Different interactions, such as synergism or competition occur within and between the populations and this modifies the growth and survival of microbes (Atlas and Bartha 1993). The life span of a building is usually several decades. Thus, there is a multitude of factors related to the development of moisture damage and attendant microbial growth.

2.1.2 Wood and concrete as building materials favoring microbial growth

Virtually any damp surface in a building, including concrete, stone, brick, plaster, wood, plastics, painted surfaces or metal, may become colonized by microbial cells settling from the air. The colonizing microbes are bacteria, fungi and some algae and together with the products of their metabolism, such as acids and polymeric materials, they form a biofilm, which can trap particulate materials, thus increasing the disfiguring effect of the biofilm (Gaylarde and Morton 1999). Wood, concrete or brick are the materials most commonly used in the building frame in the industrialized countries.

Cellulose is a major constituent of plant material and it accounts for about 30-40% of the dry weight of wood. Many microfungi are able to degrade cellulose (Dix and Webster 1995). *Alternaria, Aspergillus, Aureobasidium, Botrytis, Chaetomium, Cladosporium, Doratomyces, Exophiala, Fusarium, Gliocladium, Humincola, Mucor, Oidiodendron, Paecilomyces, Penicillium, Phialophora, Phoma, Rhinocladiella, Rhodotorula, Trichoderma*, and *Verticillium* have been reported to be among the fungi which can colonize wooden materials (Dix and Webster 1995, Viitanen and Bjurman 1995, Viitanen 1996, Gaylarde and Morton 1999, Parker et al. 1999, Reiman et al. 2000, Hyvärinen et al. 2002). *Penicillium* and *Aspergillus* species have been found to be tolerant against fluctuating humidity conditions (Viitanen and Bjurman 1995). The presence of basidiomycetes often indicates excessive moisture in a wooden structure (Levetin 1995a). In comparison of different moisture damaged building materials, the highest median concentrations of fungi and a larger variety of fungi were observed in wooden materials (Hyvärinen et al. 2002).
Numerous bacteria such as *Bacillus*, *Clostridium* and *Pseudomonas* may also colonize wood (Gaylarde and Morton 1999). Bacterial growth often occurs in wood that is either saturated with moisture or under virtually anaerobic conditions. Wood degrading bacteria have also been found together with rot fungi (Powell et al. 2001). The extent of damage varies greatly with the type of the wood; softwoods, such as pine, are generally much more susceptible than hardwoods (Higley 1995).

In addition to the ability of microbes to grow on stone surfaced materials, they may also degrade stone itself. Biodeterioration of stone by biological organisms often begins after other types of environmentally induced degradation such as weathering. Fungi require the presence of organic material which may be deposited on the surface of the stone. Fungi and bacteria produce a spectrum of inorganic and organic acids, which can demineralize various stone substrates such as calcium, iron or magnesium. Fungi are also able to degrade stone mechanically; fungal hyphae can penetrate deeply into the stone (Griffin et al. 1991). Several filamentous fungi such as *Alternaria*, *Aspergillus* spp., *A. niger*, *A. flavus*, *Aureobasidium*, *Botrytis*, *Cladosporium*, *Exophiala*, *Fusarium*, *Penicillium*, *Paecilomyces* and *Torula* contribute to deterioration of construction materials made of concrete and stone (May et al. 1993, Gaylarde and Morton 1999). Stone based materials seemed to favor the growth of *Acremonium* and *Aspergillus versicolor* (Reiman et al. 2000, Hyvärinen et al. 2002) as well as *Scopulariopsis*, *Stachybotrys*, Sphaeropsidales and *Trichoderma* (Reiman et al. 2000).

Bacteria colonizing stone may also derive energy from light and chemical redox reactions. *Thiobacillus*, *Nitrosomonas*, *Flavobacterium* and *Pseudomonas* are bacteria which have been isolated from decaying stone (May et al. 1993) and actinobacteria from stone based building materials (Hyvärinen et al. 2002).
2.2 Assessment of moisture and mold damage in buildings

2.2.1 Technical investigations of buildings

Technical investigations of moisture and mold damaged building may be divided to those bared methods like walk-troughs where no dismantling and opening of the structures are made and to those where dismantling and subsequent measurements of moisture content of a material and other such measurements are performed. Invasive investigations are rarely possible in epidemiological studies. In most case studies of indoor air quality problems, sources and location of possible moisture damage are not evident, but the analyses of the risk structures are needed anyway. Initially this is based on visual observations of moisture, mold odor or other such non-invasive methods. Investigations made by trained experts have been found to reveal more accurate results than questionnaires filled in by building occupants (Nevalainen et al. 1998). Such a walk-through based technical inspection is recommended when studying indoor air problems (Redlich et al. 1997, Dillon et al. 1999, Macher 1999, Burge et al. 2000). A grading system for moisture damage profile to support modeling of the association between excessive moisture and health consequences has been recently presented (Haverinen et al. 2002).

2.2.2 Sampling of viable indoor air microbes

The outdoor air is the most important source of indoor air fungi during frost- and snow-free periods (Burge 1990, Levetin 1995a). This is a normal phenomenon and presumably not associated with building related indoor air quality problems or health risks. Ventilation systems equipped with filters effectively remove particles from the incoming air (Reponen et al. 1989), whereas the building frame itself has been shown to act only as a poor filter against airborne particles in the ambient air (Thatcher and Layton 1995). Since it is difficult to discriminate fungi coming from outdoor and indoor sources, it is a challenge to identify the indoor sources by air sampling. Traditionally, the indoor/outdoor ratios of fungal concentrations or microbial flora have been compared (Macher 1999). Identification of microbial source with direct sampling is also commonly employed (Dillon et al. 1996, Pasanen 2001). Mold
growth is not necessarily visible in large buildings but air sampling may reveal the hidden mold growth (Miller et al. 2000, Morey et al. 2002).

Few exact guidelines have been published detailing how microbial sampling in indoor environments should be carried out. The number of samples or the sampling times needed are important factors if one aims to obtain representative results of viable fungi in indoor air. Sequential duplicate sampling for airborne viable spores has shown that their concentrations vary with time (Verhoeff et al. 1990, Waegemaekers et al. 1989). Similarly, variations in concentrations between samples taken periodically within the same week or different weeks in the same dwelling (Hunter et al. 1988, Pasanen et al. 1992, Hyvärinen et al. 2001) or office (Luoma and Batterman 2000) have been observed. In a study including 46 houses, the within-house variation in the concentrations of mold propagules was much higher than the between-house variation (Verhoeff et al. 1992). Due to these fluctuations, the decisions on where, when and how to measure biological agents are frequently based on training, experience, and the individual preferences of the investigators. Resources are generally the major limiting factor and determine how the sampling will actually be performed (Macher 1999). It has been concluded that up to eleven different days may be needed to collect sufficient data to show the presence or absence of moisture damage associated contamination with the desired degree of certainty (Hyvärinen et al. 2001). On the other hand, practical experiences have shown that even extensive air sampling protocols may not necessarily define the microbial status of a building, but other investigations such as technical inspections are still needed (Burge et al. 2000). Occupational hygiene instructions suggest that a minimum of six samples from a workplace must be taken to statistically obtain a valid assessment of the confidence interval around the mean, and a minimum of 11 samples is needed to estimate the variance of a data set (Rock 1995).

The microbes in indoor environments have traditionally been measured with culturing methods. Even though sampling viable microbes in the air reveals only about 1% of the total number of spores (Toivola et al. 2002), the advantage of the culturing based technique is related to the information on microbial genera and species obtained. On the other hand, there is no method that reveals all the characteristics of the microbial aerosol (Nevalainen et al. 1992, Crook and Sherwood-Higham 1997, Reponen et al.
New techniques, such as DNA-based or immunochemical methods for quantitative measurement and identification of different species, are being validated for indoor air applications (Haugland et al. 1999, Zhou et al. 2000, Buttner et al. 2001, Raunio et al. 2001, Calderon et al. 2002).

Impactors are commonly used for collecting culturable bioaerosols. The 1-stage impactor sampler in combination with DG18 (dichloran 18% glycerol agar) and MEA (malt extract agar) growth media was shown to give the best precision and the highest yield in terms of cfu/m³ in a comparison of five commercially available air sampling devices (Verhoeff et al. 1990). Similarly, the impactor sampler had the highest sensitivity and repeatability for fungi among several tested samplers (Buttner and Stetzenbach 1993), and was also one of the best samplers in recovering free bacteria (Jensen et al. 1992). The 2-stage impactor has even been used as a reference sampler in a comparison of the abilities of portable samplers to monitor airborne fungi (Mehta et al. 1996). The characteristics and concentrations of bioaerosol of interest determine the selection of the sampler (Nevalainen et al. 1992, Reponen et al. 2001, Pasanen 2001). The six-stage impactor, with its six collection plates, provides both a relatively large collection surface to allow screening of the different genera, and the analysis of the particle size distribution of the collected aerosol (Dillon et al. 1996).

2.3 Fungal concentrations in indoor air of schools and factors affecting them

2.3.1 Climate

The concentrations of viable microbes in school buildings have been reported in studies representing different climatic regions. Table 1 summarizes these studies, showing the location and season of the sampling, sampling device, number of the sampled school buildings and the reported mean and ranges of the fungal and bacterial concentrations. The studies are listed according to their year of publication. The reported concentrations of airborne viable fungi vary extensively, mostly depending on differences in climatic conditions. Concentrations of 1000 cfu/m³ occur in warm climates, such as southern USA and Taiwan (Dungy et al. 1986, Levetin et
al. 1995b, Su et al. 2001). In colder climates, such as Scandinavia and Canada, seasonal variations in outdoor air concentrations of fungi also affect the indoor levels of fungi. Mean concentrations of 100 cfu/m³ are found during warm seasons (Smedje et al. 1997a, Bartlett et al. 1999), but when sampling is performed during winter conditions, with snow cover on the ground, extremely low levels (10 cfu/m³) are present in the normal indoor school environment (Dotterud et al. 1995, Lappalainen et al. 2001). At that time, outdoor air concentrations are also extremely low and thus do not contribute to any major extent to the indoor mycobiota (Reponen et al. 1992). Under those circumstances, overall mean concentrations of viable airborne fungi found in school buildings are also low compared with those found in homes where concentrations of 100 cfu/m³ are often detected (Reponen et al. 1992, Hyvärinen et al. 1993).

A similar effect of climatic and seasonal variation has also been shown on microbial levels in other indoor environments. In warm or moderate regions, mean concentrations of airborne viable fungi of up to 1000 cfu/m³ have been found in office buildings (Hodgson et al. 1998, Schillinger et al. 1999, Burge et al. 2000, Pastuszka et al. 2000, Law et al. 2001). Lower number of fungi, i.e., geometric mean of 10 cfu/m³, have been found in wintertime samples in a Polish study (Pastuszka et al. 2000).

Table 1 also shows the diversity of the methods that have been used to measure the indoor air concentrations of microbes. In the 20 studies cited, 7 different sampling methods have been used. Since the collection characteristics of different sampling devices vary (Nevalainen et al. 1992, Willeke and Macher 1999), the exact levels of fungi or bacteria cannot be directly compared. All these samplers collect particles within the range 2-6 µm, which is the size range, where the most microbial particles in the indoor air are found.
Table 1. Summary of the studies of viable indoor air microbes in schools.

<table>
<thead>
<tr>
<th>Study</th>
<th>Location/ Sampling season</th>
<th>Sampling device</th>
<th>Number of sites</th>
<th>Fungal concentration</th>
<th>Bacterial concentration</th>
</tr>
</thead>
</table>
| Gravesen et al. 1983 | Denmark/ Not mentioned     | BIAP Slit-sampler | 15 schools and day-care centers | Mean 291 cfu/m$^3$ (range, 12-2000) / carpets in the rooms  
Mean 155 cfu/m$^3$ (range, 36-309) / no carpets in the rooms | Mean 1538 cfu/m$^3$ (range, 15-6000) / carpets in the rooms  
Mean 840 cfu/m$^3$ (range, 105-3000) / no carpets in the rooms |
| Dungy et al. 1986    | California/ Late spring    | Andersen multi-stage impactor | 10 schools | Mean 1040.3 spores/m$^3$                                                           | -                                                                                     |
| Thorstensen et al. 1990 | Denmark/ March            | -               | 10 schools | Mean 51 m$^3$ (range, 3-193 cfu/m$^3$)                                               | Mean 519 m$^3$ (range, 47-1429 cfu m$^3$)                                              |
| Mouilleseaux et al. 1993 | France, Paris/ Year around  | RCS             | 10 schools | Mean 100 cfu/m$^3$ (range, some units to 1000 cfu/m$^3$)                                           | -                                                                                     |
| Dotterud et al. 1995 | Norway / Winter            | BIAP Slit-sampler | 7 schools | Concentrations <30 cfu/m$^3$                                                               | -                                                                                     |
| Levetin et al. 1995b | Kansas City (KC) /Sept. Spokane (SP) / Dec. Santa Fe (SF)/ Feb. Orlando (OR)/ April | Andersen N6 sampler Burkard personal air sampler | 13 schools | Mean 1124 cfu/m$^3$(range, 136-4969 cfu/m$^3$) / KC  
Mean 130 cfu/m$^3$(range, 16-531 cfu/m$^3$) / SP  
Mean 352 cfu/m$^3$(range, 17-4134 cfu/m$^3$) / SF  
Mean 1119 cfu/m$^3$(range, 76-6454 cfu/m$^3$) / OR | -                                                                                     |
<p>| Smedje et al. 1997b | Sweden /Spring-Summer | 25-mm nucleopore filters | 38 schools | Mean 500 cfu/m$^3$ (range 100-4500 cfu/m$^3$), Relations to subjective indoor air quality | Mean 900 cfu/m$^3$ (range, 100-18000 cfu/m$^3$) |
| Wålinder et al. 1997 | Sweden / March, January | 25-mm nucleopore filters | 2 schools | Mean 580 cfu/m$^3$ (range, 60-1500 cfu/m$^3$) / low air exchange rate | Mean 1500 cfu/m$^3$ (range, 110-3600 cfu/m$^3$) / low air exchange rate |
| |  |  |  | Mean 250 cfu/m$^3$ (range, 100-600 cfu/m$^3$) / high air exchange rate | Mean 870 cfu/m$^3$ (range 80-1400 cfu/m$^3$) / high air exchange rate |
| Cooley et al. 1998 | USA (southern Atlantic states/ year around | Andersen air sampler (two stage) | 48 schools | Cladosporium mean 177 cfu/m$^3$ (complaint areas) |  |
| |  |  |  | Cladosporium mean 210 cfu/m$^3$ (non-complaint areas, lower than outdoors) |  |
| |  |  |  | Penicillium mean 60 cfu/m$^3$ (complaint areas) |  |
| |  |  |  | Penicillium mean 10 cfu/m$^3$ (non-complaint areas, higher than outdoors) |  |
| Bartlett et al. 1999 | Canada/fall, winter, spring | Andersen N6 sampler | 39 schools | GM 323 cfu/m$^3$ | GM 226 cfu/m$^3$ |
| Carlson et al. 1999 | USA, Minneapolis/ not mentioned | Andersen impactor | 1 school | Range 72-448 cfu/m$^3$, Visible mold growth | - |
| Haverinen et al. 1999a | Finland/ Not mentioned | Andersen six-stage impactor | A school center | Aspergillus versicolor range 0-180 cfu/m$^3$, Moisture damage | - |
| Rand 1999 | Canada/ Not mentioned | RCS Biotest sampler | 631 schools | Mean about 80-280 cfu/m$^3$, wood frame |  |
| |  |  |  | Mean about 50-200 cfu/m$^3$, masonry |  |
| |  |  |  | Mean about 10-50 cfu/m$^3$, steel frame |  |
| |  |  |  | Mean about 20-120 cfu/m$^3$, other frame |  |
| Robertson 1999 | USA | Andersen N6 sampler | 1 school | Trichoderma viride 494 cfu/m$^3$, Stachybotrys chartarum 212 cfu/m$^3$, Moisture damage | - |</p>
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2.3.2 Ventilation

The ventilation system influences fungal aerosol levels in school buildings. A high air exchange rate or the use of mechanical ventilation usually decreases the concentrations of microbial aerosols (Bartlett et al. 1999), partly due to filtration of incoming air, partly due to removal of particles derived from intramural sources via the exhaust air. In the rooms with low air exchange rates (0.6 ac/h), fungal (up to 1500 cfu/m$^3$) and bacterial (up to 870 cfu/m$^3$) concentrations have been reported to be twice as high compared to the rooms with a higher exchange rate (5.2 ac/h) (Wålinder et al. 1997). In naturally ventilated office buildings, the indoor fungal contents were dependent on the outdoor contents of fungi (Harrison et al. 1992) and the fungal and bacterial concentrations were both significantly higher and more variable than in an air-conditioned office (Parat et al. 1997). The highest bacterial and fungal concentrations have been detected during the starting-up period of HVAC systems, these then decrease rapidly within a few hours (Law et al. 2001, Reynolds et al. 1990).

2.3.3 Occupants’ activity and intramural sources

The presence of viable fungi in indoor air is not solely a result of the transport of the outdoor fungi, but there are also intramural sources. This can often be seen as differences in the airborne concentrations of the fungi Aspergillus sp. and Penicillium sp. (Verhoeff et al. 1992). A high level of activity by occupants has been shown to produce higher levels of spores than lower levels of activity in different indoor environments (Hunter et al. 1988, Lehtonen et al. 1993, Levy et al. 1999, Luoma and Batterman 2001). Fungal spores may be carried indoors attached to the fur of pets (Lehtonen et al. 1993) or to the clothes of the occupants (Burge 1990, Pasanen et al. 1989).

Merely the occupants’ presence in the building may affect the levels of bioaerosols. The presence of people and movement of office workers or visitors have been reflected in fluctuating numbers of airborne viable microbes (Reynolds et al. 1990, Law et al. 2001, Sessa et al. 2002). The result may be
partly explained by the resuspension of outdoor fungi previously deposited on
the floor (Buttner and Stetzenbach 1993, Levy et al. 1999). The resuspension
rate has been found to increase with particle size (Thatcher and Layton 1995)
and especially particles greater than 1 μm in particle size are resuspended
(Luoma and Batterman 2001). People have indeed been shown to be
surrounded by a “personal cloud” caused by resuspension and other factors
related to their activities (Rodes et al. 1991, Wallace 1996, Janssen et al.
2000). This can also be seen in the higher respirable particle concentrations
obtained by personal sampling than those measured by stationary sampling
techniques (Spengler et al. 1985, Clayton et al. 1993, Janssen et al. 1997,
Toivola et al. 2002).

In school buildings, the structures, furniture and textiles may act as reservoirs
of microbes. Their mechanical handling leads to the microbial emissions to the
indoor air. Gravesen et al. (1983) reported that higher indoor air
concentrations of fungi and bacteria were detected in carpeted than in non-
carpeted classrooms. Cleaning routines also affect the microbial levels in
schools (Smedje et al. 2001). The increasing age of the school building may
increase the microbial levels of indoor air (Bartlett et al. 1999, Rand 1999),
similarly as in residential buildings (Pasanen 1992). The effect of the type of
the construction of the school building on the microbial content of indoor
environment has not been studied in any great detail. Rand (1999) showed
that school buildings with steel frame had the lowest concentrations of indoor
air fungi, followed by masonry framed buildings. The wooden framed buildings
had the highest concentrations. There are a number of factors that affect
microbial content of indoor air in school environments. Since the focuses of
the related studies have been different, the studies also vary in their
conclusions.

2.3.4 Exceptional events

Exceptional events such as the water use in fire fighting may cause a
dramatic increase in the concentrations of airborne fungi. Concentrations of
viable fungi have increased up to 10000 cfu/m³ after fire fighting operations
(Morey 1993, Rautiala et al. 1996). In a 10-story office building, where massive fungal growth after fire fighting was visible, the airborne flora was dominated by *Aspergillus niger*, *A. flavus*, *A. versicolor* and *Paecilomyces* (Morey 1993). Migration of spores from water damaged-areas to non-damaged rooms was also demonstrated. Floods are another type of disastrous event leading to extensive mold growth (Morey 1996, Thi et al. 2000).

2.3.5 Moisture damage

Only a few reports deal with concentrations of viable fungi related to moisture damage in school buildings. Lappalainen et al. (2001) reported higher levels (GM=100 cfu/m$^3$) of fungi in damaged areas compared to non-damaged ones (GM=10 cfu/m$^3$). In warmer climatic conditions, where the baseline concentrations are higher due to the outdoor air spore load, it is especially difficult to detect mold damage as elevated microbial levels in the indoor air samples (Carlson et al. 1999). Although moisture and mold damage in materials present in a building are potential sources of indoor air microbes (Hunter et al. 1988, Miller et al. 2000, Ellringer et al. 2000, Backman et al. 2000, Pessi et al. 2002), the source strength of the growth may not be sufficient to increase the airborne microbial levels, especially if the baseline is high. The emissions from these types of sources are also affected by a number of factors regulating the spore release.

Regarding home environments, it has been reported that there are differences in microbial concentrations between moisture damaged and non-damaged houses (DeKoster and Thorne 1995, Pasanen et al.1992, Reponen et al. 1992, Flannigan et al. 1993, Hyvärinen et al. 1993, Pastuszka et al. 2000). These differences are more obvious during winter conditions than during seasons with higher outdoor microbial concentrations as shown in a study from daycare centers (Reponen et al. 1994). Even in temperate or tropical areas, abnormal fungal concentrations or flora may reflect difference to outdoor air despite the presence of high fungal concentrations in the outdoor air, as seen in a moisture-damaged office building (McGrath et al. 1999).
2.3.6 Release and dispersion of microbial particles

A number of factors affect the release and dispersion of the microbial spores and cells. Variation in spore release depends on the characteristics of the microbial colony and fungal spores, so that tighter colony morphology and shorter chains of spores are likely to evoke minor release (Górny et al. 2001). Thus, the release is strongly dependent on the fungal genus and species (Ingold and Hudson 1993, Pasanen et al. 1991). Some microbes such as Sporobolomyces have also active mechanisms which discharge spores into the atmosphere (Atlas and Bartha 1993). The conditions optimal for fungal growth do not always favor the release of spores, additional drying of the culture or increased temperature may be needed (Reponen et al. 1998, Adhikari et al. 1999). The release also depends on the surface where the microbial growth occurs, i.e., release is easier from rough surfaces than from smooth surfaces due to increased air turbulence above the surface. In addition, vibration facilitates the release of spores (Górny et al. 2001).

2.4 Fungal flora in indoor air of schools

The indoor air mycoflora generally largely reflects the fungal flora present in the outdoor air (Li and Kendrick 1996, Reponen et al. 1992, Wu et al. 2000) especially during frost–free periods when soil and vegetation are continuous sources of microbes. Hence, the common outdoor air fungi, Penicillium, Cladosporium, Alternaria, Aspergillus, and Aureobasidium are also among the fungi commonly found in indoor air samples of school buildings (Cooley et al. 1998, Dungy et al. 1986, Levitin et al. 1995a, Mouilleseaux et al. 1993, Rand 1999). In samples taken during winter conditions, Penicillium, Cladosporium and yeasts are the genera and groups of fungi normally found in schools (Dotterud et al. 1995, Lappalainen et al. 2001).

Certain microbes that often grow on damp building materials but do not belong to the normal mycoflora of the indoor air can be regarded as indicators
of moisture damage. These have been suggested to include *Aspergillus fumigatus*, *Aspergillus versicolor*, *Exophiala*, *Fusarium*, *Stachybotrys* and *Wallemia* (Samson et al. 1994, Flannigan and Morey 1996). The frequent occurrence of *Aspergillus versicolor* (Haverinen et al. 1999a, Backman et al. 2000, Lappalainen et al. 2001) as well as *Paecilomyces*, *Chaetomium* and *Acremonium* (Rand 1999) and *Stachybotrys* and *Trichoderma* (Robertson 1999) have been reported in the schools with moisture damage. On the other hand, the published data supporting the categorization of fungi into “normal” flora and “indicator fungi” is sparse, with differentiation more often based on empirical observations rather than on a larger database.

### 2.5 Bacterial concentrations in indoor air of schools

Bacteria that are detected in the indoor air of building environments are mainly derived from humans (Otten and Burge 1999) and thus, high concentrations of bacteria normally reflect insufficient ventilation in relation to the number of persons and activity in the space in question (Macher 1999). Mean concentrations of 100 cfu/m³ for viable airborne bacteria have been reported as normal findings in the indoor air of schools (Smedje et al. 1997a, Bartlett et al. 1999, Liu et al. 2000, Scheff et al. 2000). Levels up to 1000 cfu/m³ may occur when the air exchange rate is low (Wålinder et al. 1997) and when indoor air quality problems due to ineffective ventilation, high temperature and high relative humidity are present (Liu et al. 2000). A concentration of 5000 cfu/m³ was suggested as an upper limit of the normal range of viable airborne bacteria based on data collected from urban residences in winter conditions (Reponen et al. 1992). No association between bacterial levels and moisture damage findings was seen in a study conducted in schools (Lappalainen et al. 2001). The most common bacterial genera in the indoor air are *Micrococcus*, *Staphylococcus*, *Bacillus* and *Moraxella* (Nevalainen 1989).

In addition to the bacteria deriving from humans, several indoor air bacteria can also have environmental sources. Actinobacteria, which are mainly soil bacteria such as families of *Actinomycetaceae* and *Streptomycetaceae*...
(Stackebrandt et al. 1997), can be regarded as indicators of moisture damage
(Samson et al. 1994, Flannigan and Morey 1996). Thus, their abundant
occurrence in indoor air is a clear signal of the presence of abnormal microbial
sources in a building. A potential trend for such indication has been shown
from school environments (Lappalainen et al. 2001).

2.6 Particle size of spores and cells

Particle’s behavior in the indoor air largely depends on its size. Large
particles, e.g., those sized 10 µm or larger, settle down more rapidly than
smaller particles which may remain airborne for long periods and can be
inhaled (Owen et al. 1992). Small particles may aggregate to larger particles
and condensation also changes the size distribution towards larger particles.
The hygroscopic properties of fungal spores may vary (Pasanen et al. 1991,
Reponen et al. 1996). On the other hand, viable particles may become
nonviable and fragmented by the process of desiccation (Menetrez et al.
2001).

Particle size not only determines the fate and behavior of particles in air, but it
also greatly affects their penetration and deposition in the airways and lungs
(Seinfeld 1986, Owen et al. 1992, Venkataraman and Kao 1999). Therefore, it
is an important factor also for the health effects caused by airborne particles.
The inhaled daily doses expressed as the number of particles can be about
10^5 times higher for the fine fraction (PM_{2.5}) than for the coarse fraction (PM_{2.5-}
10) (Venkataraman and Kao 1999). Studies on outdoor air particles suggest
that especially ultrafine particles (<0.1 µm) have a major potential to cause
adverse health effects (Dockery et al. 1993, Laden et al. 2000).

The spores of different fungal generas and species vary in their shape and
size. For example, the shape of the spores of the different species of the most
common indoor air fungi, *Penicillium*, *Aspergillus* and *Cladosporium* vary from
globose to ellipsoidal and thus their spores can have distinct dimensions 2.5-
8.0 µm (*Penicillium*), 1.5-6.5 µm (*Aspergillus*) and 2-17 µm (*Cladosporium*)
(Samson *et al.* 1996). The particle sizes of microbes, which are based on measurements of cell dimensions under a microscope, do not necessarily correspond to the aerodynamic particle sizes (Pasanen *et al.* 1991; Reponen *et al.* 1996, Reponen *et al.* 1998). A six-stage impactor yields data on particle size distribution, though also fragmented particles or parts of microbes may occur in air as shown in the studies of Menetrez *et al.* (2001) and Kildeso *et al.* (2000).

It is evident that particle size distribution may vary in environments with different emission sources (Görny *et al.* 1999). The largest differences in concentrations of viable fungi between moisture damaged dwellings and non-damaged ones have been found in the size range 1.1-3.3 µm (Reponen *et al.* 1994, Hyvärinen *et al.* 2001), while in day care centers, the clearest difference was found in the size range of 3.3-4.7 µm (Reponen *et al.* 1994). The average mean diameters \(d_{g,\text{ave}}\) for fungi showed larger mean spore sizes in moisture-damaged homes than in reference homes, whereas no such difference was observed in the day-care centers (Reponen *et al.* 1994, Reponen, 1995). The reason for this variation in spore sizes is not known. A comparison of the fungal spore sizes of outdoor and indoor air revealed that average particle sizes for the most common fungi were larger in the outdoor air (Mishra *et al.* 1997).

The size of bacterial cells and spores is usually around 1 µm, thus being smaller than that of fungal spores. There may well be differences in the particle size distributions of bacteria in different indoor environments. The highest concentrations of viable airborne bacteria in new suburban homes were in the size range of 1.1-2.1 µm, while in moisture damaged homes, the highest levels were detected in the size range 2.1-3.3 µm (Nevalainen 1989).
2.7 Symptoms in relation to school environment

2.7.1 Symptoms among schoolchildren

An association between moisture and mold damage in buildings and adverse health outcomes has been shown in a number of questionnaire studies from residential and work environments (Dales *et al.* 1991, Spengler *et al.* 1994, Maier *et al.* 1997, Peat *et al.* 1998, Bornehag *et al.* 2001). The relatively few studies suggest that this association is also true in school environments. A higher prevalence of respiratory symptoms, respiratory infections and other symptoms, such as eye irritation and fatigue have been reported among schoolchildren exposed to moisture and mold in schools compared with children attending the reference school (Haverinen *et al.* 1999a, Savilahti *et al.* 2000, Åhman *et al.* 2000). Visits to physician and the use of antibiotics were more prevalent among children in a moisture-damaged school than in a non-damaged one (Savilahti *et al.* 2000). A high prevalence of asthma (13%) was reported among the children in a moisture damaged school (Haverinen *et al.* 1999a), compared to the general asthma prevalence among Finnish primary schoolchildren of 4.4% (Timonen *et al.* 1995). The results concerning the link between schoolchildren’s asthma and fungal concentrations of indoor air in the school have been somewhat conflicting. Smedje *et al.* (1997a) found a positive correlation between asthma prevalence among schoolchildren and the concentrations of viable fungi and bacteria in the school environment, while no difference in the fungal exposure between asthmatic or non-asthmatic schoolchildren was noted in the study by Su *et al.* (2001).

School-aged children spend about 20% of their time in school (Schwab *et al.* 1992, Statistics Finland, 1992) and 58% at home (Schwab *et al.* 1992). Thus, it is obvious that in addition to the school environment, the exposure received in the home environment may also play a role in the health outcomes. There is some preliminary evidence that moisture and mold exposure occurring both at school and at home trigger the manifestations. This was seen as increased
asthma prevalence among schoolchildren (Taskinen et al. 1997) and as increased IgG levels to some fungi (Hyvärinen et al. in press).

2.7.2 Symptoms among school personnel

Health outcomes in moisture and mold damaged schools have also been shown among teachers and other school personnel. Such symptoms include fatigue, headache, runny and stuffy nose, eye irritation, nausea, sleeping difficulties, episodes of fever, dry throat and hoarseness (Thörn et al. 1996, Cooley et al. 1998, Sigsgaard et al. 2000, Åhman et al. 2000).

Responses to the exposure in the moisture and mold damaged school environment have been verified by objective clinical measurements. An increased production of proinflammatory mediators in the nasal lavage fluid was reported among the school personnel working in a school with moisture damage (Hirvonen et al. 1999). The responses disappeared during vacation, but increased again by the end of the fall term, thus pointing to a connection between the school environment and the inflammatory responses in nasal lavage fluid. In addition, reduced nasal patency measured by acoustic rhinometry and increased levels of lavage biomarkers have been shown among teachers (Norbäck et al. 2000b, Wålinder et al. 2001), as well as increased mucosal reactivity to histamine (Rudblad et al. 2001) and decreased pulmonary function measured as FVC and FEV₁ (Dahlqvist and Alexandersson 1993).

2.7.3 Exposure aspects

Although the association between moisture damage of buildings and adverse health effects is apparent, the factors responsible for the symptoms are not at all clear (Bornehag 2001). Many authors have linked microbial findings in the indoor air of school buildings with the health complaints of building users. Cooley et al. (1998) showed that in the certain areas of the school buildings where people complained of symptoms, the indoor air concentrations of Penicillium and Aspergillus were higher compared to the concentrations in the
outdoor air. Elevated levels of *Stachybotrys* and *Trichoderma* (Robertson 1999) or *Aspergillus versicolor* (Haverinen et al. 1999a) have been associated with adverse health outcomes. Li et al. (1997) showed an association between elevated *Aspergillus* levels and work related symptoms in day-care centers. The evidence that elevated levels of fungi would be a causal factor for the health complaints remains insubstantial. The role of volatile organic compounds (VOC), mycotoxins or other factors related to microbes may have importance with respect to the health effects (Ström et al. 1994, Johanning et al. 1996, Etzel 2000), but these factors have rarely been studied in connection with school buildings.

There are multitudes of other factors contributing to symptoms. In a Swedish study, the increased asthma prevalence among schoolchildren seemed to be attributable to technical and physical parameters, i.e., larger school size, classrooms with more open shelves, lower room temperature and higher relative humidity as well as to the higher concentration of formaldehyde (Smedje et al. 1997a). Even low socioeconomic status, determined by parental occupation, may be a risk factor for reduced lung function among schoolchildren (Demissie et al. 1996).

2.7.4 Perceived indoor air quality

Personal perceptions can be used to characterize the conditions of the indoor environments. Smedje et al. (1997b) reported that 53% of the personnel of Swedish schools perceived the indoor air quality as poor. They found that the perception of poor air quality associated with elevated levels of VOCs, total molds, bacteria, and respirable dust. Complaints of dustyness in schools have been associated with an increased number of particles larger than 1 μm (Kinshella et al. 2001). High temperature causes a sensation of dryness, independently of the air humidity (Reinikainen and Jaakkola 2001). Personal characteristics can affect the perception; e.g., young, female and persons with atopic background and poorer general health condition may be more sensitive (Skov et al. 1987, Sundell and Lindvall 1993, Norbäck 1995, Smedje et al. 1997b, Wargocki et al. 1999, Moschandreas and Chu 2002).
Perceptions of unpleasant smells, dustiness and dirtiness may be associated with moisture damage, since there were fewer complaints after the repair of moisture damage in a school building (Rudblad et al. 2001). The occupants’ environmental perceptions were also improved after renovation of the ventilation system and changing the carpeting materials (Pejtersen et al. 2001).

2.8 Effects of interventions on indoor air quality and health

2.8.1 Effect of moisture damage repairs on microbial status of the building

Assuming that moisture damage causes abnormal presence of microbial spores in the indoor air, the renovation and elimination of such a source should decrease the numbers of microbes in the air. There are examples of successful mitigation. An abnormal fungal profile in the indoor air with elevated concentrations of *Penicillium* was shown to normalize and become similar to the profile in the outdoor air after the renovation in schools (Cooley et al. 1998) and also in a hotel building (Ellringer et al. 2000). Reynolds et al. (1990) reported a major reduction in the total concentration of viable airborne fungi from a level >7200 cfu/m³ to the level of 50 cfu/m³ after the repair of a leak in the roof and the cleaning of the ventilation system in an office.

Moisture damage renovation of a daycare-center resulted in a significant decrease in the concentration of airborne (1→3) β-D-glucan, a cell wall component of fungi and some bacteria (Rylander et al. 1997). Shaw et al. (1999) reported a reduction in the indoor concentration of VOCs after moisture damage repairs in houses. Thus, there is some evidence of decreasing levels of indoor air pollutants as a result of renovations aimed at the elimination of their sources.
2.8.2 Moisture and mold damage repairs in relation to the health of occupants

As stated earlier, there is a well-documented association between moisture and mold damage and adverse health effects experienced by occupants (see paragraphs 2.7.1 and 2.7.2). Assuming that these health effects are reversible, renovation of the moisture damage should lead to an improvement in the symptoms. Such changes have been documented in a few studies. A decrease in respiratory symptoms and infections among schoolchildren following water damage renovation has been reported (Haverinen et al. 1999b, Savilahti et al. 2000). In a Swedish study, where the association between health outcomes and damage findings was more obvious among teachers than among students, the decrease in symptom prevalence after renovation was also more obvious in the teaching staff (Åhman et al. 2001). Increased prevalence in fatigue, headache, eye irritation, dry throat, hoarseness, cough, and dyspnea reported by teachers disappeared after remedial actions in a school study in the USA (Cooley et al. 1998). Similar findings concerning nose and eye irritation, headache and sinusitis among teachers were found in a Danish study (Sigsgaard et al. 2000).

On the other hand, partial moisture damage repairs may not be sufficient to decrease the elevated symptom prevalence, as observed in some case studies. In a study of an office building, the health problems disappeared only after extensive and thorough repair of the moisture damage (Andersson et al. 1993). According to Jarvis and Morey (2001), after thorough repair measures in a moldy building, re-entry of occupants with hypersensitivity disease, originally due to the building related exposure was possible. Instead, the high frequencies in fatigue, headache and stuffy nose among pupils were still found after the repairs in the moisture-damaged school suggesting incomplete repairs (Åhman et al. 2001). Also, the increased prevalence of mucous membrane irritation among teachers even one year after remedial measures (Rudblad et al. 2001) evidenced for the insufficient elimination of emission sources. Only a slight and non-significant decrease in symptom prevalence was reported in a small group of workers in a moisture and mold-damage day-
care center after renovation (Rylander et al. 1997). However, a small decrease in airway responsiveness was found in a pulmonary function test.

An improvement in the health status of occupants may be achieved by their transfer into a non-damaged environment. This was shown among office workers by Sudakin (1998) and Johanning et al. (1999). Koskinen et al. (1995) reported a decrease in respiratory symptoms and infections among children after they left a mold-damaged day-care center.

2.8.3 Other technical measures

Several building related factors may contribute to the environmental perceptions as well as the symptoms experienced by the occupants. Increasing the ventilation effectiveness by renovating the HVAC-system has been shown to reduce the asthmatic symptoms of schoolchildren (Smedje and Norbäck 2000) as well as the symptoms and complaints of the indoor air quality among the teachers (Jalas et al. 2000, Mathisen and Frydenlund 2000). A lower frequency of general symptoms and less irritation of the mucous membranes were also intervention-associated findings. Likewise, after the installation of a ventilation system, which provided the office workers the possibility to individually control the temperature and airflow, significantly lower frequencies of symptoms, i.e., skin, eye, nose and throat irritation, were observed compared with a reference group of employees (Menzies et al. 1997). When a casein-containing flooring cover was an obvious source of indoor air quality problems in apartment houses, increasing the ventilation efficiency did not decrease the symptoms of the occupants, but the removal of the harmful component turned out to be necessary (Stridh and Andersson 1995). Reduced complaints of indoor air quality among office workers were observed after both increasing the ventilation efficiency and removing the highly polluting materials to low-emission materials (Pejtersen et al. 2001). After removing carpets from classrooms, improvement in general symptoms among schoolchildren was evident (Mathisen and Frydenlund 2000).
In an office building with poor ventilation system, both the levels of respirable suspended particulate matter and occupants’ symptoms reduced simply by increasing the efficiency of cleaning (Kemp et al. 1998). The elimination of an old carpet, found to be a source of VOCs in an office, decreased the prevalence of headache and increased productivity of the employees (Wargocki et al. 1999). After installing high efficiency particulate air filters into the ventilation system serving the main living room, somewhat lower levels of airborne microorganisms were demonstrated but no improvement in the asthmatic symptoms of occupants were detected (Warburton et al. 1994). The relatively short time spent in the living room probably masked the potential benefit. The electrostatic air cleaning system decreased the concentrations of the indoor air particles and also the children’s absenteeism in day-care centers (Rosén et al. 1999).

As the examples given above indicate, the elimination of identified sources of indoor air pollution may have beneficial effects on the occupants' health. This suggests that the symptoms in question are reversible. This also supports the hypothesis that the pollution source has a causal relationship with the health outcomes, although the underlying mechanisms responsible for the symptoms are still poorly understood.
3 AIMS OF THE STUDY

This research aimed to characterize different factors affecting the microbial quality of indoor air in school buildings, to provide information about the importance of moisture damage in school buildings as a risk factor for schoolchildren's symptoms, and to document the changes in microbial exposure and symptom prevalence among children as a result of moisture and mold renovation.

The detailed objectives of this study were:

1. to characterize fungal concentrations in school buildings and to identify the most important building related factors affecting them (I and II)

2. to investigate how moisture damage affects the concentration and flora of viable indoor air microbes in schools (I and II)

3. to characterize the size distributions of indoor microbes with respect to moisture damage in concrete and wooden schools (III)

4. to determine whether the moisture damage of a school building is associated with symptoms among schoolchildren (I and IV)

5. to investigate the effects of moisture and mold renovations on microbial indoor air quality and the prevalence of respiratory and general symptoms among the schoolchildren (IV)
4 MATERIAL AND METHODS

4.1 Study protocol

In studies I-III, technical investigations and microbial characterization were performed in 32 school buildings located in central Finland. The schools were either primary or secondary schools owned by the municipalities. The effects of moisture damage were studied by classifying the buildings into moisture damaged (index) and non-damaged buildings (reference) according to the observations made during the technical investigations. The effect of building frame material was studied separately. On an average, the school buildings that had a timber frame were older than the schools that had a frame made of concrete or brick. The numbers of school buildings classified as index/reference and wooden/concrete buildings in the studies I-IV are presented in Table 1.

<table>
<thead>
<tr>
<th></th>
<th>Index</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concrete/Brick</td>
<td>Wooden</td>
</tr>
<tr>
<td>Studies I-III</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Study IV</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

The prevalence of respiratory symptoms was studied in 26 schools (study I). The total number of pupils was 5345. Six out of 32 schools were excluded from the epidemiological analyses, since the symptom questionnaire used in these schools was slightly different from that used for the rest of the schools.

The effect of moisture and mold renovation of schools on microbial exposure and children’s health was studied in two school buildings (A_{int} and B_{int}) of concrete construction (study IV). Two reference schools (A_{ref} and B_{ref}) of concrete construction without such damage were included in the study. These buildings were included in the material that consisted of 32 schools in studies
I-III. The sampling campaigns as well as the questionnaire surveys were performed before and after repair measures in the damaged schools and at the same time in their reference schools.

4.2 Technical investigations of the schools

The classification of the school buildings was based on technical investigations, which were performed at the beginning of the studies in all the buildings. Trained civil engineers thoroughly inspected the buildings without dismantling or opening the structures according to a standardized protocol developed earlier (Nevalainen et al. 1998). A detailed checklist was used for recording various types of moisture signs in the building. Surface moisture recorders (Doser BD-2) were used to assess the moisture level of surface materials. The types of and obvious reasons for the damage were recorded when possible. The areas and severity of the damage as well as the size of the building were taken into account when classifying the schools into damaged and non-damaged buildings. This classification was used in the analysis of microbial and health data.

4.3 Characterization of microbial indoor air quality of schools

Indoor microbes were sampled by using six-stage impactors (Andersen 10-800). Samples for airborne fungi were taken simultaneously on 2% malt extract agar (MEA) and on dichloran 18% glycerol agar (DG18), and samples for bacteria on tryptone glucose yeast agar (TGY). All the samples were taken in winter and during the school days when the buildings were occupied. Sampling times were from 7 to 15 minutes and detection limits ranged from 2 to 5 cfu/m$^3$ depending on the sampling time. The numbers of air samples taken on different growth media in the schools in each campaign are presented in Table 2. From 5 to 22 samples per sampling campaign were taken in each school, mainly from the rooms occupied by children and teachers i.e., classrooms, hall facilities and personnel rooms. Each room was sampled once in studies I-III. In the intervention study (IV), samples were taken twice in the same rooms, i.e., before and after intervention. In addition,
10 outdoor air samples were taken. The mean number of samples taken in corresponding index and reference schools and in schools in the intervention study (IV) were similar.

Table 2. Total numbers of samples taken from indoor air of the schools.

<table>
<thead>
<tr>
<th>Studies I-III</th>
<th>Index</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>Wooden</td>
</tr>
<tr>
<td>MEA</td>
<td>117</td>
<td>54</td>
</tr>
<tr>
<td>DG18</td>
<td>117</td>
<td>37</td>
</tr>
<tr>
<td>TGY</td>
<td>117</td>
<td>52</td>
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</table>

<table>
<thead>
<tr>
<th>Study IV *</th>
<th>$A_{int}$</th>
<th>$B_{int}$</th>
<th>$A_{ref}$</th>
<th>$B_{ref}$</th>
<th>total n</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEA</td>
<td>2x18</td>
<td>2x16</td>
<td>2x17</td>
<td>2x13</td>
<td>148</td>
</tr>
<tr>
<td>DG18</td>
<td>2x18</td>
<td>2x16</td>
<td>2x17</td>
<td>2x13</td>
<td>148</td>
</tr>
<tr>
<td>TGY</td>
<td>2x18</td>
<td>2x16</td>
<td>2x17</td>
<td>2x13</td>
<td>148</td>
</tr>
</tbody>
</table>

* duplicate sampling performed before and after the renovation in the damaged schools

Fungi were incubated for 7 days at 25°C, and bacteria for up to 14 days at 20°C. The total number of bacterial colonies was counted after 5 days of incubation, actinobacteria colonies were incubated for 14 days. The concentrations were counted as colony forming units per cubic meter of air (cfu/m$^3$) using positive hole correction (Andersen 1958). The fungi were identified morphologically by genus using an optical microscope. *Aspergillus fumigatus*, *A. glaucus*, *A. niger*, *A. ochraceus*, *A. penicillioides* and *A. versicolor*, were identified to the species level. *Aspergillus fumigatus*, *A. penicillioides*, *A. versicolor*, *Altemaria*, *Eurotium*, *Exophiala*, *Fusarium*, *Mucor*, *Phialophora*, *Sporobolomyces*, *Stachybotrys*, *Trichoderma*, *Ulocladium*, *Wallemia* and actinobacteria were considered as indicators of moisture damage in further data analysis (Samson et al. 1994, Flannigan and Morey 1996). The detection of actinobacteria colonies was based on their dry, actinobacteria-type appearance.
4.4 Assessment of the ventilation type and the age of the building

First, the analyses to study the effect of the ventilation type and the age of the building were performed separately for two construction types, wooden and concrete (II). The age of the building and the ventilation type were also associated with the frame type, so most of the wooden buildings were older (built between 1890-1975) than the concrete buildings (1935-1994). Likewise, most of the wooden schools had natural ventilation (73% of the studied rooms) and most of the concrete buildings mechanical exhaust and air supply (63%). Thus, the additional analyses to study the effect of these characters were performed combining the schools of both frame types.

4.5 Follow-up of respiratory symptoms

Detailed information on respiratory symptoms and general health of the participating children was collected by a questionnaire. The questionnaire used was a modified version of those used in other Finnish studies on respiratory symptoms and diseases (Susitaival and Husman 1996). The questionnaire consisted of 32 questions concerning health, perceived indoor air quality in school and home environment characteristics. Questionnaires were delivered to the schools, where teachers distributed them to the pupils and then collected the completed forms. Secondary school pupils answered the questionnaire by themselves. Parents were asked to fill in the questionnaire together with the children in primary schools. The number of participating children was 4365 in the study I, and a total of 1371 and 1330 children aged from 7 to 17 years, participated in the study before and after the intervention, respectively (IV).

4.6 Statistical methods

Concentrations of airborne microbes were not normally or log-normally distributed and therefore, non-parametric tests were used for data analysis. Differences in total concentrations of viable airborne fungi and bacteria and
concentrations of the most common fungi between index and reference schools were compared with Wilcoxon Rank-Sum test (studies I, II, IV) and those between the intervention and reference schools with Mann Whitney’s U-test (IV). $\chi^2$ – test was used to test for the differences in the occurrence of certain fungal genera between the buildings (I, II, IV) and McNemar test for variation within the building (IV). Kruskal-Wallis oneway analysis of variance was used to test differences in microbial concentrations and particle size distributions between the index and reference schools of similar construction. Multiple comparisons were performed using Dunn’s test (Zar 1996) (I-III). The effect of the ventilation type and the age of school buildings were examined with mixed model analysis of variance. When studying the effect of the ventilation type, the data were adjusted for moisture damage and when studying the age of the building, adjusting was made for the ventilation type and moisture damage.

The association between symptoms and moisture damage findings in index and reference schools was analyzed using logistic regression models. Crude odds ratios were calculated after cross-tabulations as well as differences in symptom prevalence before and after intervention within a school and between the schools using $\chi^2$ – test. Odds ratios were adjusted for gender, age, atopy and moisture observations at home. Associations between symptoms and moisture damage repair in damaged schools were verified using logistic regression models adjusting for gender, age, moisture observations at home, atopy and smoking (I and IV).

SAS statistical package (SAS Institute Inc. 1990) was used for all analyses in studies I-III and and SPSS statistical package, version 10 (SPSS inc., 1988) for the analyses in study IV, where all the differences were tested using exact p-values.
5 RESULTS

5.1 Moisture damage in schools

Technical investigations on moisture damage revealed several types of damage in the school buildings (II, Figure 1). Eight out of 32 schools were considered non-damaged. There were no notable differences in the mean relative humidity of the indoor air and temperature between the school buildings.

5.2 Airborne viable fungi in school buildings

5.2.1 Distributions of fungal concentrations

The geometric means (GM) and ranges of total concentrations of airborne viable fungi and bacteria and those for actinobacteria in the indoor air of the school buildings are presented in Table 3. After classifying the school buildings according to the moisture damage observations carried out in the technical inspections, no significant difference in concentrations of fungi between the index and reference schools was found (Table 3, column A) (I).

When the buildings were classified according to the frame construction material, higher (p<0.05) mean concentrations of fungi were detected in the wooden schools than in the concrete schools (Table 3, column B; II, Figure 4). The analyses of the frequencies of different concentration categories (II, Figure 5a-d) showed the following differences between the building types:

- values below the detection limit (<DL) were only found in the schools of concrete construction
- in the wooden schools, the lowest detected concentration was 5 cfu/m³
- frequency of low values (1 to 50 cfu/m³) was 60-70% in concrete schools, 50% in wooden schools (p<0.001)
the concentrations 50-200 cfu/m$^3$ were almost three times more frequent in the wooden schools (41%) than in the concrete schools (16%) (p<0.001)
- concentrations higher than 500 cfu/m$^3$ were rare, but more frequent (p=0.031) in the wooden schools than in concrete schools

Moisture damage-associated differences in the fungal concentrations were observed in the concrete schools; total concentrations of fungi were significantly higher (p<0.05) in the index schools than in the reference schools. In the wooden schools, no such difference was found (Table 3, column C; I, Figures 1 and 2). When the statistical variation of total concentrations in the wooden and concrete schools with and without moisture damage was considered, intra-school variances were greater than inter-school variances in all cases except in the reference schools of wooden construction. The greatest intra-school variance was found in concrete reference schools (II, Table 1).

The following features were typical for the concentration distributions in the concrete schools: (II, Figure 5a-b):

- values <DL were less frequent (p=0.001) in the index schools (6%) than in the reference schools (25%) (p<0.001)
- concentrations 50-200 cfu/m$^3$ were more common in the index schools (18%) than in their references (10%) (p=0.125)
- concentrations 200-500 cfu/m$^3$ were found equally often (4-6%) in the index and reference schools

In the wooden schools, the only difference was the more common occurrence of concentrations from 200 to 500 cfu/m$^3$ in the index schools (13%) than in the reference schools (5%) (Figure 1; II, Figure 5c-d).
Table 3. Geometric means (GM), arithmetic means (AM) and ranges of concentrations of airborne viable microbes as well as p-values of significance of differences between the two groups of the schools (I-III). Column A, the schools classified as moisture damaged (index) and reference schools; column B, classified into wooden and concrete schools; and column C, wooden and concrete schools classified according to the moisture damage.

<table>
<thead>
<tr>
<th>Fungi</th>
<th>A</th>
<th></th>
<th>B</th>
<th></th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>Index</td>
<td>Ref.</td>
<td>Wooden</td>
<td>Concrete</td>
<td>Wooden</td>
</tr>
<tr>
<td>GM</td>
<td>cfu/m³</td>
<td>cfu/m³</td>
<td>cfu/m³</td>
<td>p</td>
<td>cfu/m³</td>
</tr>
<tr>
<td>AM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
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<td>ND-550</td>
<td>N.S.</td>
<td>5-950</td>
<td>ND-510</td>
</tr>
<tr>
<td>N</td>
<td>325</td>
<td>101</td>
<td>129</td>
<td>297</td>
<td>91</td>
</tr>
<tr>
<td>GM</td>
<td>26</td>
<td>18</td>
<td>57</td>
<td>16</td>
<td>57</td>
</tr>
<tr>
<td>AM</td>
<td>94</td>
<td>60</td>
<td>99</td>
<td>41</td>
<td>102</td>
</tr>
<tr>
<td>Range</td>
<td>ND-950</td>
<td>ND-550</td>
<td>N.S.</td>
<td>5-950</td>
<td>ND-510</td>
</tr>
<tr>
<td>Bacteria</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>169</td>
<td>53</td>
<td>72</td>
<td>150</td>
<td>52</td>
</tr>
<tr>
<td>GM</td>
<td>593</td>
<td>432</td>
<td>844</td>
<td>447</td>
<td>985</td>
</tr>
<tr>
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<td>903</td>
<td>1359</td>
<td>983</td>
<td>1552</td>
</tr>
<tr>
<td>Range</td>
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<td>ND-5900</td>
<td>N.S.</td>
<td>48-11400</td>
<td>ND-7600</td>
</tr>
<tr>
<td>Actinobact.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>155</td>
<td>53</td>
<td>72</td>
<td>136</td>
<td>52</td>
</tr>
<tr>
<td>GM</td>
<td>2.3</td>
<td>1.3</td>
<td>5.9</td>
<td>0.9</td>
<td>5.7</td>
</tr>
<tr>
<td>AM</td>
<td>28</td>
<td>4</td>
<td>58</td>
<td>2.8</td>
<td>76</td>
</tr>
<tr>
<td>Range</td>
<td>ND-2700</td>
<td>ND-47</td>
<td>N.S.</td>
<td>ND-2700</td>
<td>ND-43</td>
</tr>
</tbody>
</table>
| N number of samples
p refers to statistical significance of differences
ND not detected
N.S. not statistically significant
5.2.2 Fungal flora in the indoor environment of the school buildings

The most common fungi in the indoor air of the school buildings were *Penicillium*, yeasts, *Cladosporium*, and *Aspergillus*. These genera accounted for about 70% of the mean total concentration of airborne viable fungi in the wooden schools, and approximately 60% in the concrete schools (II, Figure 7a-d). The rank order of the fungal types was the same on all the six stages of the six-stage impactor (III, Table 3). Concentrations of *Penicillium* (p<0.0001), yeasts (p<0.0208) and *Cladosporium* (p<0.0002) were higher in the wooden schools than in the concrete schools. The following genera were also more frequent in the wooden schools: *Oidiodendron*, *Olpidichium*, *Paecilomyces*, *Hyalodendron*, *Wallemia* and Sphaeropsidales-group (II, Figures 6a-b). *Aspergillus versicolor* was more frequent (p=0.03) in the concrete schools than in wooden schools.

The effect of moisture damage was seen in the concrete schools as elevated concentrations of *Cladosporium* (p<0.05). The fungi that were more frequently detected in the index schools than in their reference schools and the fungi that were not detected in the reference schools at all are presented in Table 4.

Table 4. Fungi detected more frequently in the index schools than in their reference schools. Asterisk (*) indicates fungi, that were not detected in the corresponding reference schools at all.

<table>
<thead>
<tr>
<th>Wooden index schools</th>
<th>Concrete index schools</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acremonium *</td>
<td>Cladosporium</td>
</tr>
<tr>
<td><em>Aspergillus versicolor</em> *</td>
<td>Penicillium</td>
</tr>
<tr>
<td>Stachybotrys *</td>
<td>yeasts non-sporing isolates</td>
</tr>
<tr>
<td></td>
<td><em>Acremonium</em> *</td>
</tr>
<tr>
<td></td>
<td><em>Aspergillus versicolor</em></td>
</tr>
<tr>
<td></td>
<td>Geomyces *</td>
</tr>
<tr>
<td></td>
<td>Exophiala *</td>
</tr>
<tr>
<td></td>
<td>Mucor *</td>
</tr>
<tr>
<td></td>
<td><em>Oidiodendron</em> *</td>
</tr>
<tr>
<td></td>
<td><em>Scopulariopsis</em> *</td>
</tr>
<tr>
<td></td>
<td>Stachybotrys *</td>
</tr>
</tbody>
</table>
5.3 Airborne viable bacteria in school buildings

Significantly higher (p=0.0032) concentrations of airborne bacteria were detected in the wooden schools than in the concrete schools (I, Figure 2). Also, the concentrations of actinobacteria alone were higher (p=0.0001) in the wooden schools than in the concrete schools. (Table 3).

Moisture damage did not have any effect on the mean concentrations of total viable bacteria in either school type. In the concrete schools, actinobacteria were more prevalent and their concentrations were higher (p<0.05) in the index schools than in the references (Table 3, column C). In the wooden schools, no difference was found between the index and reference schools.

5.4 The effect of the ventilation type and the age of the building

When the additional analyses to study the effect of the ventilation type on airborne fungal levels were performed, this effect seemed to be significant in combined analysis, i.e., when both the wooden and concrete buildings were combined. Since some of the buildings had parts, which were either mechanically or naturally ventilated, the analysis was performed room by room. The significance was seen as lower concentrations (p<0.0001) of airborne viable fungi in the rooms with totally mechanical ventilation. No major difference in the fungal levels between the rooms with natural ventilation and mechanical exhaust was detected either in separate (II, Figure 2) or in combined analysis for both construction types of buildings. The number of the rooms with totally mechanical ventilation was too low (1%) in the wooden buildings for the comparison of the effect of ventilation separately in wooden and concrete schools.

In the separate analyses for the two frame types of school buildings, the effect of the age of the buildings on fungal aerosol levels was not significant (II, Figure 3). In the combined analyses of the both frame types of buildings, the highest levels were found in the two oldest groups of buildings and the lowest
levels in the youngest buildings, but the overall effect of the building age on the concentrations of viable airborne fungi was not statistically significant.

5.5 Fungi in wintertime outdoor air samples

The geometric mean (GM) of total concentration of airborne viable fungi was 5.9 cfu/m³ (range <DL-18 cfu/m³) in wintertime outdoor air samples (n=10). The most common fungi were Penicillium, non-sporing isolates and yeasts found in 60%, 40% and 20% of the samples, respectively. Their concentrations remained low, smaller than 7 cfu/m³ in each sample. Other microbes, e.g., Aspergillus, Paecilomyces, Scopulariopsis and Oidiodendron, were only detected as single colonies in sporadic samples.

5.6 Effect of moisture damage repairs on the microbial indoor air quality of the school buildings

The GMs and ranges of total concentrations of airborne viable fungi and bacteria and those for actinobacteria before and after the repairs are presented in Table 5. In the initial survey, before any repair measures were carried out in the damaged schools, the GMs of total concentrations of airborne fungi were higher in the intervention school A_int than in the reference school A_ref (p<0.001/p=0.005 depending on sample media; IV, Figure 1). Values below the detection limit (DL) were less frequent (p<0.001-0.006) in the intervention schools A_int and B_int (0-3%) than in the reference schools A_ref and B_ref (25-31%) (IV, Figure 1).

The total number of fungal types (groups, genera, species) found in the air samples were 22-25 in the two damaged and 9-14 in the reference schools. In all, 15 fungal genera found in the intervention schools were not detected in the reference schools. With respect to fungi regarded as moisture damage indicators, Mucor, Exophiala and Stachybotrys occurred in the damaged schools but were not found in the reference schools. Eurotium and Wallemia were also more frequently detected in the index schools.
After the moisture damage renovation was completed in the intervention school $A_{\text{int}}$, a significant decrease in the mean concentrations of viable airborne fungi ($p=0.002$) was observed (Table 5; IV, Figure 1). The frequencies of samples with low levels became similar to those measured in the reference school $A_{\text{ref}}$ (IV, Figure 1). All observed fungal concentrations were $<100$ cfu/m$^3$. Likewise, the number of the microbial types was at the same level than in $A_{\text{ref}}$ (IV, Table 3). *Mucor* and *Wallemia* disappeared and a lower frequency of *Eurotium* was found after renovation in the intervention school $A_{\text{int}}$.

In the partly repaired intervention school $B_{\text{int}}$, the mean fungal concentration was higher ($p=0.010$) in the final survey than before the repairs and higher ($p<0.001$) than in its reference school $B_{\text{ref}}$ ($p<0.001$) (Table 5; IV, Figure 1). *Stachybotrys* disappeared but *Eurotium* and *Trichoderma* were more frequent in the final survey compared with the initial sampling (IV, Table 3).

Wider ranges of total concentrations of airborne bacteria were observed in the two damaged schools than in the two reference schools in the initial study, although no difference was found in the mean concentrations between the schools. After the thorough renovation of the school $A_{\text{int}}$, the total concentration of viable bacteria were significantly lower ($p=0.006$) than before the repairs. In the intervention school $B_{\text{int}}$, the mean concentration of bacteria was significantly higher ($p<0.001$) after partial repairs than before (Table 4).
Table 5. Geometric means (GM), arithmetic means (AM) and ranges of concentrations of airborne viable microbes before and after the interventions in the two pairs of schools, (IV).

<table>
<thead>
<tr>
<th></th>
<th>$A_{int}$</th>
<th></th>
<th>$A_{ref}$</th>
<th></th>
<th>$B_{int}$</th>
<th></th>
<th>$B_{ref}$</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>final</td>
<td></td>
<td>Initial</td>
<td>final</td>
<td></td>
<td>Initial</td>
<td>final</td>
</tr>
<tr>
<td></td>
<td>Cfu/m$^3$</td>
<td>cfu/m$^3$</td>
<td>P</td>
<td>cfu/m$^3$</td>
<td>cfu/m$^3$</td>
<td>p</td>
<td>cfu/m$^3$</td>
<td>cfu/m$^3$</td>
</tr>
<tr>
<td><strong>Fungi</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>34</td>
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<td>32</td>
<td>26</td>
</tr>
<tr>
<td>GM</td>
<td>23</td>
<td>6.3</td>
<td></td>
<td>6.1</td>
<td>7.9</td>
<td>19</td>
<td>23</td>
<td>8.7</td>
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<tr>
<td>AM</td>
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<td>ND-54</td>
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<td>4-130</td>
<td>ND-120</td>
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<td><strong>Bacteria</strong></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>18</td>
<td>18</td>
<td></td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>13</td>
</tr>
<tr>
<td>GM</td>
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<td></td>
<td>239</td>
<td>277</td>
<td>429</td>
<td>1455</td>
<td>367</td>
</tr>
<tr>
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<td>830</td>
<td>672</td>
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<tr>
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<td>0.006</td>
<td>ND-2100</td>
<td>ND-2600</td>
<td>N.S.</td>
<td>54-2000</td>
<td>150-3900</td>
</tr>
<tr>
<td><strong>Actinobact.</strong></td>
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<td>0.2</td>
<td></td>
<td>0.1</td>
<td>0.1</td>
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<tr>
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<td>ND-4</td>
<td>ND-3</td>
<td>ND-8</td>
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</tr>
</tbody>
</table>

N number of samples
p refers to statistical significance of differences
ND not detected
N.S. not statistically significant
5.7 **Size distributions of indoor air microbes in schools**

The total concentrations of airborne viable fungi were higher in the wooden schools than in the concrete schools through all size classes from 0.65 to >7.0 µm (p<0.001). Moisture damage-associated differences in the size distributions were seen in the concrete schools; concentrations of viable fungi in the size class of 1.1-2.1 µm (stage 5) were higher (p<0.05) in the index schools than in the reference schools. In the wooden school buildings, no such a difference was found (III, Figure 1).

The average geometric mean diameter (d_{g,ave}) of total viable fungi was smaller (p<0.001) in wooden schools than in concrete schools, but variation according to genus was observed. When comparing the buildings for the presence of moisture damage, d_{g,ave} of both total fungi and the most common fungal types were almost invariably smaller in the index schools than the reference schools of both construction types, although the difference was significant (p<0.05) only for *Penicillium* spores in the concrete schools (III, Table 4).

The mean concentrations of viable airborne bacteria were significantly higher (p<0.001-0.034) in the wooden schools than in the concrete schools. This difference was observed in the particle size ranges of 0.65-2.1 µm and 4.7- >7.0 µm (stages 5-6 and 1-2, respectively). (III, Figure 2).

No differences in particle size distributions of airborne bacteria were observed between the index and reference schools of the two construction types. The highest proportions of actinobacteria were detected on stage 6 (0.65-1.1 µm).
5.8 Prevalence of moisture damage-associated respiratory symptoms among schoolchildren

Respiratory symptoms were more prevalent among the children in the index schools than among the children in the reference schools (I, Table 2/column A). Likewise, after classifying the buildings according to the frame material, a higher symptom prevalence was found among the children in the index schools of concrete/brick construction than among the children in the corresponding reference schools (I, Table 2/column B). A similar trend was observed in the association with moisture damage in wooden schools, but the differences were generally not significant (I, Table 2/column C).

A difference in the symptom prevalence between the damaged and reference schools was also seen in the initial survey of the intervention study (IV). The differences in 9 out of 12 symptoms were significant (p<0.009) between the intervention school A_{int} and its reference school A_{ref}. In the intervention school B_{int}, the prevalences of hoarseness and general symptoms were significantly higher than in its reference school B_{ref} (IV, Table 4).

Children perceived the indoor air quality to be poor significantly more often in index schools than in reference schools (I, Table 3/column A). More complaints about indoor air characteristics came from children in index schools of both concrete/brick and wooden construction compared with their reference schools (I, Table 3/-columns B and C).

After the renovation of the intervention school A_{int}, a decrease (p<0.036) in the prevalence of 10 out of 12 symptoms was observed. The differences in the symptom prevalence between the intervention school A_{int} and the reference school A_{ref} disappeared (IV, Table 4).

The prevalence of rhinitis, sore throat and cough with phlegm in spring term were lower (p<0.034) after the repairs than before the repairs in the intervention school B_{int}. Separate analyses were made for the 74 children who
took part in both the initial and the final survey in the school B_{int}. No improvement of reported symptoms of these 74 individuals were found, except for an improvement in the of fall term reports of difficulties in concentration (IV, Table 5).

After the renovation in the intervention school A, a significant reduction in reports of weekly occurring annoyance factors was reported. In the intervention school B, mold odor was reported less often after repairs, but draft (p=0.019) and dust and dirt (p=0.005) were even more often reported than before repair measures were undertaken in the school (IV, Table 6).
6 DISCUSSION

6.1 Fungal concentrations

When the school buildings with different frame materials, i.e., concrete/brick or wood, were grouped together, no significant differences in the concentrations of viable airborne fungi between the 24 moisture damaged schools and 8 reference schools were found. The building frame material greatly influenced the fungal concentrations. The mean concentrations of fungi were significantly and systematically higher in the wooden schools than in the concrete schools. A similar difference between building frame type and airborne spore load has also been reported by Rand (1999). On the other hand, in this study, the presence of moisture damage did not increase the fungal concentrations in the wooden schools, whereas the moisture damage significantly increased the fungal concentrations in the buildings of concrete/brick construction. An association between moisture damage and total concentrations of fungi in school environment has also been shown in the study of Lappalainen et al. (2001). Some results on the association between fungal concentrations and moisture damage in school buildings are ambiguous, probably due to the strong effect of outdoor air fungi on the indoor air in these studies (Levetin et al. 1995b, Carlson and Quraishi 1999).

In general, fungal concentrations detected in the indoor air of schools were low (GM 9-58 cfu/m³) compared to those previously found in Finnish homes (GM 58-150 cfu/m³) (Reponen et al. 1992, Hyvärinen et al. 1993). Lower levels in schools compared to residential environments have also been reported in another study from Northern conditions (Dotterud et al. 1995). This is probably due to larger volume of rooms and thus the greater spatial dilution in the schools than in the residences. In addition, there are less normal fungal sources in the schools than in homes.

Analyses for intra- and inter-school variation of fungal concentrations showed that about 60% of the variation was explained by variation within the school
buildings. A categorized comparison of concentrations in the concrete schools showed that findings under the detection limit were more common in the reference schools, and values from 50 cfu/m$^3$ to 200 cfu/m$^3$ in the damaged schools. High values, exceeding 200 cfu/m$^3$, were sporadically observed even in reference schools, without any association with moisture damage. Normal background sources of spores, such as human activities or transport of spores on clothing of occupants probably explain these unusually high concentrations (Hunter et al. 1988, Pasanen et al. 1989, Lehtonen et al. 1993, Luoma and Batterman 2001). Thus, moisture damage in school buildings is not necessarily characterized by clearly “high” concentrations of fungi in this cold climate, but rather as an elevation of the base level. This elevation of the base level concentration was only detected in the concrete buildings, while in the wooden school buildings, moisture damage did not alter the mean fungal concentrations. Only a greater proportion of concentrations from 200 cfu/m$^3$ to 500 cfu/m$^3$ in the damaged wooden schools was different from their reference schools.

The effect of the ventilation type and the age of the building on fungal concentrations were also analyzed. This was made room by room according to the ventilation type in each of them. Totally mechanical ventilation decreased the fungal levels. Measurements on air exchange rates in schools have indicated similar findings (Wålinder et al. 1997, Bartlett et al. 1999). In this study, the air exchange measurements were not carried out and the number of rooms with mechanical ventilation was too low for separate analyses in the wooden school buildings. Thus, the effect of ventilation cannot be analyzed in more detail. Interestingly, the age of the building had no significant effect on fungal levels, opposite to the findings of Pasanen (1992), Bartlett et al. (1999) and Rand (1999). It is apparent that several factors significantly affect the microbial concentrations in the indoor air and cannot be totally distinguished from each other.
6.2 Fungal flora

The most common fungal genera or groups were similar in both frame types of school buildings, i.e., Penicillium, yeasts, Cladosporium and Aspergillus. These are also the most common fungi in residential environments in northern climate (Reponen et al. 1994, Pasanen et al. 1992). Their common occurrence in indoor air is explained by their ubiquity in nature and outdoor air in Finland (Reponen et al. 1994). They even dominate the indoor air during the wintertime conditions when outdoor levels are low. The same fungal genera have also been found in other school studies in Northern countries (Dotterud et al. 1995, Lappalainen et al. 2002). While the rank order was the same, the concentrations of Penicillium, yeasts and Cladosporium were higher in the wooden schools than in the concrete schools, suggesting that the wooden frame may act as a source of these fungi. Differences in the frequency of less common fungi between the school buildings of different frame type were also seen. Aspergillus versicolor was more common in the concrete schools than in the wooden schools, while Oidiodendron, Olpitrichium, Paecilomyces, Hyalodendron, Wallemia and Sphaeropsidales-group were more abundant in the wooden schools. Interestingly, Hyvärinen et al. (2002) found that ceramic products including concrete products and bricks favored the growth of Aspergillus versicolor. They also have found a larger diversity of microbes in moisture damaged wooden materials compared with the other materials. The growth of various microbes on both wooden and stone based materials is possible (May et al. 1993, Dix and Webster 1995, Viitanen and Bjurman 1995, Viitanen 1996, Gaylarde and Morton 1999, Parker et al. 1999, Reiman et al. 2001) and because there are many other building materials found in both types of buildings, i.e., concrete or wooden frames, no detailed conclusions on the association of individual genera and building type can be drawn.

Moisture damage elevated concentrations of Cladosporium (>10 cfu/m$^3$) in the concrete schools. Cladosporium spp. has also been found in higher concentrations in damp residences (Pasanen et al. 1992). Thus, elevated levels of Cladosporium may be an indication of moisture damage. Likewise
the more frequent prevalence of *Penicillium* and yeasts associated with moisture damage in concrete schools. These fungi have also been found to be the most frequent genera growing on damaged building materials (Hyvärinen *et al.* 2002). These associations were only observed in concrete buildings, and it appears that there were also other sources for these fungi than the moisture damage in the wooden school buildings.

*Aspergillus versicolor*, *Stachybotrys* and *Acremonium* were associated with moisture damage for both frame types and *Exophiala, Mucor, Geomyces, Scopulariopsis* and *Oidiodendron* in the concrete schools. Airborne *Stachybotrys, Acremonium* and *Oidiodendron* have also been found in residences and day care centers with mould problems (Hyvärinen *et al.* 1993), and *Aspergillus versicolor* in other studies in moisture damaged schools (Haverinen *et al.* 1999a, Lappalainen *et al.* 2001).

There were no differences in average concentrations of viable airborne bacteria in school buildings categorized either by the construction type or by the moisture damage. Indoor air bacteria originate mainly from humans and high concentrations of viable airborne bacteria usually indicate that there is insufficient ventilation in a building (Nevalainen 1989, Macher 1999). Thus, measurements of total airborne viable bacteria do not seem to provide information about the possible presence of moisture damage. The occurrence of actinobacteria, which are bacteria not of human origin but frequently growing in damaged building materials (Hyvärinen *et al.* 2002), can be regarded as a sign of moisture damage in concrete schools. Actinobacteria have also been found to have such indicator value in residences (Nevalainen *et al.* 1991).

The geometric mean of total concentration of airborne viable fungi in wintertime outdoor air samples was low, 5.9 cfu/m$^3$, consisting mainly of *Penicillium*, non-sporing isolates and yeasts. This is a considerably lower level than that observed in the indoor air of school buildings during the wintertime. Thus, fungi in wintertime outdoor air seem only to have marginal contribution to the levels in the indoor air of schools. Considering the source strength of
other factors such as mold damage, outdoor air fungi seem to have no practical importance. As Reponen et al. (1992) have previously shown, the outdoor concentrations of microbes in the Scandinavian winter conditions during the snow cover are low, and indoor measurements mainly reflect the microbial content of the indoor environment. In milder climates, the effects of snow cover cannot be exploited, and the indoor concentrations of fungi are affected by the outdoor air fungi throughout the year (Dungy et al. 1986, Levetin et al. 1995a).

6.3 Effects of moisture damage renovation on microbial indoor air quality

After the building had undergone a thorough renovation, both the levels of airborne microbes and the fungal diversity of the samples decreased significantly, down to the levels detected in the reference school or even lower. Obviously the elimination of the moisture and mold damage which were the abnormal sources of fungi, had been successful. Normalization of fungal indoor air concentrations after moisture damage renovation has also been reported by Cooley et al. (1998), Haverinen et al. (1999b), Ellringer et al. (2000) and Reynolds et al. (1990). A decrease in other contaminants, e.g., (1→3) β-D-glucans or volatile organic compounds has been shown in the studies by Rylander et al. (1997) and Shaw et al. (1999). In the other intervention school, where the repairs were only partial and the total elimination of moisture damage failed, there was even an increase of contamination detected in the air samples. Thus, microbial concentrations in the indoor air seem to follow the presence or elimination of moisture damage, evidently acting as a surrogate of the exposure in question.

6.4 Particle size distributions of fungi in schools

The highest concentrations of fungi were in the size range of 1.1-4.7 μm in both the wooden and concrete schools. In concrete schools, moisture damage
was associated with higher concentrations of airborne fungi within the size range of 1.1-2.1 µm. Differences between moisture-damaged and non-damaged residences and day-care centers have been previously presented in the size ranges of 1.1-2.1 µm, 2.1-3.3 µm and 3.3-4.7 µm (Hyvärinen et al. 2001, Reponen et al. 1994). The discrepancy in aerodynamic particle sizes may be due to differences in the buildings and their use. Occupant density, activities and ventilation rates, which are different in different types of buildings, can affect spore release and resuspension of fungi via air currents and vibration (Görny et al. 2001).

The average mean diameters \(d_{g,\text{ave}}\) for total viable fungi and the most ubiquitous fungi were smaller in the moisture damaged schools than in the reference schools of both construction types. The differences in fungal flora or different sources are the most likely explanations. The finding contrasts with the reports about larger mean spore sizes in moisture-damaged homes (Reponen et al. 1994) while no difference in the mean spore sizes between damaged and reference day-care centers was observed (Reponen 1995). Hence, no general conclusion on whether the moisture damage increases or decreases the mean size of airborne fungi can be drawn at this point.

6.5 Symptoms

The existence of moisture damage in the school building was a risk factor for respiratory symptoms among schoolchildren. The prevalence of respiratory symptoms among the schoolchildren was higher in the damaged than in the non-damaged schools. Similar relationships have been reported for school-aged children living in mold-damaged residential environments (Dales et al. 1991, Brunekreef et al. 1992, Spengler et al. 1994, Koskinen et al. 1999). Differences in the symptom prevalence during the spring season were greater than during the fall, which may be an evidence for the prolonged exposure period of the entire school year. The association between moisture damage and respiratory symptoms was also only statistically significant in the concrete schools, whereas in the wooden schools the trend was similar but did not achieve statistical significance.
Indoor characteristics causing discomfort were more often reported in the damaged schools than in the reference schools. Symptomatic children complained more than non-symptomatic children both in the moisture damaged and reference schools. Perceived indoor air quality has been shown to characterize the indoor environmental conditions in schools (Smedje et al. 1997b) and ill health obviously leads to the more sensitive perceptions of the discomfort factors (Norbäck 1995, Smedje et al. 1997b).

The symptom prevalence decreased remarkably after the thorough renovation of the school building. A significant decrease was observed in the prevalence of 10 symptoms out of the studied 12 symptoms, thus supporting earlier findings among schoolchildren after repairs were undertaken in a damaged school (Savilahti et al. 2000). These clear and measurable changes in symptom prevalence as a result of moisture damage renovation also again are evidence in favour of a causative relationship between the damage and symptoms, although the actual exposing agents are still obscure. The positive effect of building renovation emphasizes the importance of building maintenance in the prevention of adverse respiratory health outcomes. In order to be effective, the renovation must eliminate the microbial sources. Obviously this was not achieved in the other school, where only a partial repair was attempted. Some improvement in the symptom prevalence was also observed there. An insufficient elimination of moisture and mold damage neither lead to the hoped-for result in another school study (Åhman et al. 2000).

No change in symptom prevalence was found among those children in the final survey who had attended the damaged school before the repair measures were attempted. The final survey was carried out one year after the partial repairs had been completed. Apparently, for the children who had been exposed to the damaged school environment before the repairs and developed symptoms, one year was not long enough to permit any recovery. Hence, the main result was that even partial repairs appeared slightly beneficial for the new pupils in that school, while the already symptomatic
children did not enjoy this benefit. Consistently, Jarvis and Morey (2001) observed that chest symptoms among adult occupants in a mold damaged office remained elevated for several months after they had left the building.

After the complete renovation, a significant reduction in reports of weekly occurring annoyance factors was seen. Instead, the perceived quality of the indoor air was poorer after the partial repairs, possibly this being indicative of inadequate ventilation. Obviously, the successful repairs led to good perceived indoor air quality. Resolving indoor air problems often necessitate both improvement of ventilation and elimination of the emission sources (Stridh and Andersson 1995) as was the case in the intervention school in this study where the school underwent a thorough renovation.
7 CONCLUSIONS

This investigation concerned the effects of moisture damage, and the effects of repairs of such damage on the microbial quality of the indoor air of school buildings and on schoolchildren’s health. Microbial exposure was characterized by measurements of viable fungi and bacteria from indoor air of school buildings, and the status of the schoolchildren’s health was surveyed by questionnaires. The following conclusions can be drawn from the results of this study:

1. The type of building frame material affected the microbial content of the building; mean concentrations of fungi were significantly higher in the school buildings of wooden construction than in the schools with a concrete/brick frame. This difference was mainly attributable to the higher concentrations of the common fungi *Penicillium*, yeasts, *Cladosporium*, and *Aspergillus*.

2. An association between concentrations of fungi and moisture damage was found in concrete schools, but not in wooden schools nor in the combined material of schools. Typically, in moisture-damaged school buildings of concrete construction, the geometric mean wintertime concentration was above 10 cfu/m$^3$, there was a low frequency of samples with values under the detection limit, and a frequent occurrence of samples with concentrations above 50 cfu/m$^3$.

   Elevated concentrations of *Cladosporium* and actinobacteria (concrete schools) and the occurrence of *Aspergillus versicolor*, *Stachybotrys* and *Acremonium* (both frame types of schools) were associated with moisture damage.

3. In moisture damaged concrete schools, higher levels of fungi were observed especially in the particle size class of 1.1-2.1 µm. In the wooden
school buildings, no moisture damage-associated differences in the size distributions of indoor air microbes were seen.

4. Moisture damage in the school building was a significant risk factor for respiratory symptoms among schoolchildren. The association between moisture damage and respiratory symptoms was statistically significant in the concrete schools, while only a trend towards such an association was seen in the wooden schools.

5. After a thorough renovation, the levels of airborne microbes and the fungal diversity of the samples normalized to the level in the reference school. However, after only partial repairs, an increase of contamination was detected in the air samples.

A remarkable decrease in symptom prevalence among schoolchildren was achieved by thoroughly renovating the moisture- and mold-damaged school building. A less marked improvement was seen in the school which underwent only partial repair measures.
8 REFERENCES


EFA, European Federation of Asthma and Allergy Associations. 2001. Indoor air pollution in schools, the project report, Allergy and Asthma Federation, Helsinki, Finland, pp. 178.


Macher J (Ed.). 1999. Bioaerosols: Assessment and Control, American Conference of Governmental Industrial Hygienists, Cincinnati, Ohio, USA.


building and a naturally ventilated building over one year. *Atmospheric Environment* 31(3), 441-449.


School office, City of Kuopio, oral information, 25.3.2002.


SPSS inc. 1988. SPSS-X™ user’s guide, 3rd Edition, Chicago, IL, USA.


