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

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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/m^3, there was a low frequency of samples with values under the detection limit, and the 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. 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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ABBREVIATIONS

ac/h	air change per hour
a_w	water activity
CFU	colony forming units
$d_{g,ave}$	average mean diameter
DG18	dichloran 18% glyserol agar
DL	detection limit
DNA	deoxyribonucleic acid
FEV_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

LIST OF ORIGINAL PUBLICATIONS

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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 restrains. 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 ^{o}C (optimum 22-27^{o}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, Humicola, 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.*

2001). 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/m^3 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/m^3 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/m^3 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/m^3) 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/m^3 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/m^3 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/m^3, 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.

Study	**Location/ Sampling season**	**Sampling device**	**Number of sites**	**Fungal concentration**	**Bacterial concentration**
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	Mean100 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	-

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 250 cfu/m^3 (range, 100-600 cfu/m^3) / high air exchange rate	Mean 1500 cfu/m^3 (range, 110-3600 cfu/m^3) / low 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	-

Lee and Chang 2000	Hong Kong/ November-January	A portable air sampler for agar plates (Burkard)	5 class-rooms	-	Mean <1000 cfu/m^3 , lower than outdoors
Liu *et al.* 2000	Southeastern US/April and May	Andersen N6 sampler	2 schools	-	Mean 77-1463 cfu/m^3 , median 64-1359 cfu/m^3 (range 10-4400 cfu/m^3), Perceived IAQ problems
Scheff *et al*. 2000	Illinois/ February	Andersen N6 sampler	1 school	Range of mean concentrations, 460-811 cfu/m^3	Range of mean concentrations, 577-946 cfu/m^3
Lappalainen *et al*. 2001	Finland/ Winter	Andersen six-stage impactor	9 schools	GM 42 cfu/m^3 (range 5-95) non-damage area GM 97 cfu/m^3 (range 35-780) damage area GM 132 cfu/m^3 (range 25-405) most damaged area	GM 256 cfu/m^3 (range, 10-4400) non-damaged GM 457 cfu/m^3 (range, 10-4600) damage area GM 538 cfu/m^3 (range, 75-3500) most damaged area
Smedje and Norbäck 2001	Sweden/ Winter-Spring	25-mm nucleopore filters	39 schools	GM 200 cfu/m^3 (range 30-4500 cfu/m^3)	GM 360 cfu/m^3 (range 50-18000 cfu/m^3)
Su *et al*. 2001	Taiwan /winter, summer	Burkard sampler	2 schools	GM 9730 cfu/m^3 winter GM 3565 cfu/m^3 summer	

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/m^3 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, Levetin *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/m^3 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/m^3 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/m^3 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 genera 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 Kildesø *et al.* (2000).

It is evident that particle size distribution may vary in environments with different emission sources (Górny *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 ($d_{g,ave}$) 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 FEV_1 (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/m^3 to the level of 50 cfu/m^3 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) ß-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 1. Numbers of school buildings included in the studies.

	Index		**Reference**	
	Concrete/ Brick	**Wooden**	**Concrete/ Brick**	**Wooden**
Studies I-III	12	12	3	5
Study IV	2		2	

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.

		Index		Reference		
Studies I-III		**Concrete/ Brick**	**Wooden**	**Concrete/ Brick**	**Wooden**	**total n**
	MEA	117	54	34	19	224
	DG18	117	37	29	19	202
	TGY	117	52	33	20	222

Study IV *		**A_{int}**	**B_{int}**	**A_{ref}**	**B_{ref}**	
	MEA	2x18	2x16	2x17	2x13	148
	DG18	2x18	2x16	2x17	2x13	148
	TGY	2x18	2x16	2x17	2x13	148

* 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*, *Alternaria, 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). χ^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 χ^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/m^3
- frequency of low values (1 to 50 cfu/m^3) 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.

	A			**B**			**C**					
				Wooden	**Concrete**		**Wooden**			**Concrete**		
	Index cfu/m^3	**Ref. cfu/m^3**	**P**	**cfu/m^3**	**cfu/m^3**	**p**	**Index cfu/m^3**	**Ref. cfu/m^3**	**p**	**Index cfu/m^3**	**Ref. cfu/m^3**	**p**
Fungi												
N	325	101		129	297		91	38		234	63	
GM	26	18		57	16		57	58		19	9	
AM	94	60		99	41		102	92		41	40	
Range	ND-950	ND-550	N.S.	5-950	ND-510	<0.05	5-950	12-550	N.S.	ND-330	ND-510	<0.05
Bacteria												
N	169	53		72	150		52	20		117	33	
GM	593	432		844	447		985	565		473	366	
AM	1168	903		1359	983		1552	860		998	929	
Range	ND-11400	ND-5900	N.S.	48-11400	ND-7600	0.0032	48-11400	81-2300	N.S.	ND-7600	ND-5900	N.S.
Actinobact.												
N	155	53		72	136		52	20		103	33	
GM	2.3	1.3		5.9	0.9		5.7	6.3		1.3	0.1	
AM	28	4		58	2.8		76	10		3.5	0.3	
Range	ND-2700	ND-47	N.S.	ND-2700	ND-43	0.0001	ND-2700	ND-47	N.S.	ND-43	ND-7	<0.05

N number of samples
p refers to statistical significance of differences
ND not detected
N.S. not statisticaly 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, Olpitrichum, 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.

Wooden index schools	**Concrete index schools**
Acremonium *	*Cladosporium*
Aspergillus versicolor *	*Penicillium*
Stachybotrys *	yeasts
	non-sporing isolates
	Acremonium *
	Aspergillus versicolor
	Geomyces *
	Exophiala *
	Mucor *
	Oidiodendron *
	Scopulariopsis *
	Stachybotrys *

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/m^3 (range <DL-18 cfu/m^3) 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/m^3 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_{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_{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_{ref} (IV, Table 3). *Mucor* and *Wallemia* disappeared and a lower frequency of *Eurotium* was found after renovation in the intervention school A_{int}.

In the partly repaired intervention school B_{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_{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_{int}, the total concentration of viable bacteria were significantly lower ($p=0.006$) than before the repairs. In the intervention school B_{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).

	A_{int}			**A_{ref}**			**B_{int}**			**B_{ref}**		
	Initial Cfu/m^3	**final cfu/m^3**	**P**	**Initial cfu/m^3**	**Final cfu/m^3**	**p**	**initial cfu/m^3**	**Final Cfu/m^3**	**p**	**initial cfu/m^3**	**Final cfu/m^3**	**p**
Fungi												
N	36	36		34	30		32	32		26	26	
GM	23	6.3		6.1	7.9		19	23		8.7	2.1	
AM	31	16		23	14		26	37		55	5.6	
Range	ND-130	ND-96	0.002	ND-250	ND-54	N.S.	4-130	ND-120	0.010	ND-510	ND-25	<0.001
Bacteria												
N	18	18		16	16		16	16		13	13	
GM	888	210		239	277		429	1455		367	103	
AM	1868	765		721	830		672	1857		580	296	
Range	71-7600	ND-4700	0.006	ND-2100	ND-2600	N.S.	54-2000	150-3900	<0.001	50-1500	21-1600	0.013
Actinobact.												
N	18	18		16	16		16	16		13	13	
GM	0.6	0.2		0.1	0.1	N.S.	1.2	-		-	0.4	
AM	1.6	0.2		0.2	0.2		2.4	-		-	1.2	
Range	ND-10	ND-4	N.S.	ND-4	ND-3		ND-8	-	-	-	ND-22	-

N number of samples
p refers to statistical significance of differences
ND not detected
N.S. not statisticaly 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 ($p \leq 0.009$) 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/m^3) compared to those previously found in Finnish homes (GM 58-150 cfu/m^3) (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*, *Olpitrichum, 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) ß-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 (Górny *et al.* 2001).

The average mean diameters ($d_{g,ave}$) 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.

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