

MICROBIAL EXPOSURE, SYMPTOMS AND INFLAMMATORY MEDIATORS IN NASAL LAVAGE FLUID OF KITCHEN AND CLERICAL PERSONNEL IN SCHOOLS

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Abstract

Objectives: The aim of this study was to investigate how the microbial conditions of kitchen facilities differ from those in other school facilities. The health status of the personnel was also studied. **Materials and Methods:** The microbial investigations were conducted in six moisture-damaged schools and two reference schools. The symptoms of the kitchen personnel were surveyed with questionnaires and inflammatory responses in nasal lavage (NAL) fluid were measured. **Results:** The total concentrations of airborne microbes were lower in kitchens than in other facilities of the schools. However, the occurrence of moisture damage increased the airborne microbial concentrations both in kitchens, and in other facilities. Bacterial concentrations were high on surfaces in the damaged kitchens. Gram-negative bacteria predominated, but also thermophilic bacteria and mycobacteria were detected. Respiratory and general symptoms were prevalent both among kitchen workers and clerical personnel in the moisture-damaged environments. Reported allergies and repeated respiratory infections were connected with high IL-4 concentrations in NAL fluid. Median concentrations of studied inflammatory mediators (NO, IL-4, IL-6 and TNF- α) were slightly higher in NAL samples of kitchen workers than among the clerical personnel. **Conclusions:** Kitchen facilities differ from other facilities of the school building for their moisture conditions and microbial contamination. Thus, they represent a specific type of environment that may affect the health status of the personnel.

Key words:

Fungi, Bacteria, Indoor air, Moisture damage, Health survey, Nasal lavage fluid

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INTRODUCTION

The association between building dampness and adverse health effects has been shown in many epidemiological studies in residential environments [1]. This association has also been found in school environments [2–4]. Reasons for damage are typically flaws in design or construction, aging of materials or insufficient maintenance [5]. Particularly in school kitchens, the conditions are favorable to create even severe moisture damage. Moisture load is remarkable during cooking, dishwashing and cleaning of floors and other surfaces. This sets particular demands for ventilation, building materials and structures. If moisture barriers fail, intrusion of water into the structures and consequent microbial growth are inevitable. Elevated dampness also leads to occurrence of other biological agents such as dust mites [6,7]. In 2002, 24 new cases of occupational skin diseases and 16 occupational respiratory diseases were reported among kitchen workers in Finland [8]. It can be hypothesized that the occupational diseases were at least partly caused by moist conditions and subsequent microbiological exposures in the kitchens.

So far, only few studies have dealt with microbial conditions in institutional kitchens. In a hospital kitchen, where the personnel had respiratory symptoms, airborne fungal concentrations were considered normal [9]. In household kitchens, enterobacteria [10,11], coliform bacteria and other gram-negative rods [12,13] were found prevalent on moist surfaces like those around the sink. It is still unclear, how structural moisture damage in kitchens and associated microbial growth contributes to the microbial status of the indoor air in these facilities.

The health of occupants of different indoor facilities has usually been evaluated with symptom questionnaires. Among methods used to find clinical evidence for the responses to microbially altered environments, measurements of inflammatory mediators such as nitric oxide (NO) and interleukin-6 (IL-6) in nasal lavage (NAL) fluid have been shown to be a promising approach. In several studies in moisture-damaged buildings, a link between the exposure and these responses and the increased prevalence of symptoms has been shown [14–16].

The aim of this study was to investigate how the microbial status of kitchen facilities differs from that in other school facilities and how the moisture damage alter this status in kitchen facilities. The presence of storage mites was also screened. The health status of the personnel was studied with symptom questionnaires and with determinations of inflammatory mediators in NAL fluid.

MATERIALS AND METHODS

Description of school buildings

Eight school buildings, located in Central Finland and erected during 1950–1990, were studied. The school buildings were inspected for visible signs of moisture by trained civil engineers and the visual observations of moisture were verified with a surface moisture recorder (Doser Bd-2) as described earlier [5]. The school building was considered damaged if there was extensive moisture and mould damage needing replacement of surface covering or opening, drying or renewing of structures. This classification was also applied to the kitchen facilities: in the six moisture-damaged schools, kitchens were also damaged, while in the kitchens of two reference schools, only minimal signs of moisture damage were detected [17]. Cleaning methods and volume of water used for cleaning (mean, 5.3 l/m²/day) were similar in all the kitchens. About 70% of the total volume of water was used for floor washing and the remaining 30% for dishwashing and cooking. The ventilation system was mechanical exhaust in three moisture-damaged kitchens and mechanical supply and exhaust in the others.

Microbial investigations

Air sampling. Air samples were used to study fungal and bacterial concentrations and microbial biota in the kitchen facilities and in other parts of the school buildings. In addition to the kitchen, lunchroom of the school and coffee room of kitchen personnel were included in the kitchen facilities, comprising a total of 3–5 samples per school. Other facilities of the schools included classrooms, corridors, teachers' rooms and gyms, where 6–17 samples per school were collected, depending on the size of the

building. Air sampling was carried out in wintertime when snow covered the ground to minimize the effect of outdoor air microbes. Air samples were collected using an Andersen six-stage impactor (10-800; Graseby Andersen, Atlanta, GA, USA) with sampling time of 15 min. Fungal samples were collected on 2% malt extract agar (MEA, Biokar, Beauvais, France) with added chloramphenicol and on dichloran 18% glycerol agar (DG18, Oxoid, Basingstoke, England, glycerol from BDH, Poole England) with added chloramphenicol, and bacterial samples on tryptone yeast extract glucose agar (TYG, BD Difco, Le Pont de Claix, France) with added natamycin. Fungal colonies were counted and identified after 7 days incubation at 25°C. The fungi were identified morphologically to genus by light microscopy (Labophot-2, Nikon, Tokyo, Japan). *Aspergillus fumigatus*, *A. versicolor*, and *A. niger* were identified to species level. Total numbers of bacterial colonies were counted after 5 days of incubation (20°C) and the number of actinomycete colonies was counted from the bacteria plates after 14 days.

Surface and material sampling. In the kitchens, microbial flora was also characterized by taking swab samples from visibly damaged surfaces and from sites where high moisture content had been detected with the surface moisture recorder. Damaged surfaces were detected both in moisture-damaged kitchens (26 sites) and in reference kitchens (8 sites), and these samples were combined in the statistical analyses. Reference samples were taken from undamaged but otherwise similar surfaces. Undamaged sampling sites also included an external wall under the window and an inverted funnel above the cooking device in each kitchen. All the surface samples were taken with a sterile swab and transported to the laboratory in buffer solution (distilled water with 42.5 mg/l $\text{KH}_2\text{PO}_4 \cdot 7\text{H}_2\text{O}$, 250 mg/l MgSO_4 , 8 mg/l NaOH and 0.02% Tween 80 detergent). The sampling area varied from 50 to 100 cm². Fungal samples were cultured on 2% MEA and DG18, and bacterial samples on TYG. Incubation conditions were constant with air samples. For more specific characterization of bacterial populations, 21 surface samples were cultured also on TYG treated at 70°C water bath for 10 min before culture, and on eosin methylene blue agar

(EMB, BD BBL, Le Pont de Claix, France) with added natamycin (35°C, 1 day), from which 59 bacterial isolates were collected. In addition to the surface swab samples, six material samples from moisture-damaged sites were also taken for microbial analyses. The samples, 1–5 g, were cut into little pieces and extracted into the buffer solution described above. Suspensions were held in an ultrasonic bath for 30 min and placed in a shaker for 60 min. Culture procedure was the same as presented for surface samples (2% MEA, DG18, TYG, TYG at 70°C, EMB). From material samples, two thermophilic bacteria were isolated. The bacterial isolates from surface and material samples were typed and identified by using colony morphology and routine bacteriological test (gram staining, catalase and oxidase tests, biochemical tests and API-kits).

Mycometer sampling and analyzing technique was used to detect possible mould growth on damaged surfaces ($n = 15$) in four kitchens. Swab samples were collected by rubbing a 9-cm² surface area with a moist sterile cotton swab [18]. The activity of the enzyme β -N-acetylhexosaminidase (the Mycometer®-test) was determined by contacting the swab with a synthetic enzyme substrate for 30 min. When hydrolyzed, fluorophore is released, which can be quantified using fluorometry (The Mycometer Handbook, September 2001). The enzyme activity is expressed in fluorescence count, which is then categorized into one of three empirically derived categories [19].

Mycobacteria. Mycobacteria were cultured from 8 surfaces and 6 material samples. For mycobacterial analyses of surfaces, the swabs were shaken into the dilution buffer and inoculated to egg medium containing nystatin (100 mg/l) and supplemented with glycerol (pH 6.3) (i) and Na-pyruvate (pH 6.3) (ii) [20], and to R2A medium (Difco, Detroit, Michigan, USA) (iii). To destroy more rapidly growing microbes of the sample, two ml of the original suspension was decontaminated with 0.7 M NaOH followed by 5% oxalic acid [21] and another two ml with 0.05% cetylpyridinium chloride (15 min + 15 min centrifugation) [21,22]. Fifty μl of the resuspended sediment and its tenfold dilution were inoculated to media (i) and (ii) these media adjusted to pH 5.5 (iv, v) [20], (iii) and Mycobacteria 7H11 agar (Difco, Detroit, Michigan,

USA) containing OADC enrichment (10%) and nystatin (100 mg/l) (vi). From the material samples, mycobacteria were detached into the dilution buffer containing nystatin (100 mg/l) as described in the "Surface and material sampling" sub-section and cultured as described above. All cultures were grown at 30°C for 3 months. Acid-fastness of colonies was checked by Ziehl-Neelsen staining, and the acid-fast isolates were analyzed for fatty acids and alcohols to verify them as mycobacteria [23].

Dust mites. Dust samples for mite analyses were collected on fibreglass filters (MN 640 W) by vacuuming the kitchen floors for 20–40 sec and/or an upholstered chair for 45 sec. These samples were stored at –20°C before analyses. Altogether 18 samples were collected. From each sample, two sub-samples of 25–50 mg dust were taken for counting and identification of mites. After clearing the mites in lactic acid, they were picked out under a stereomicroscope at low magnification (x13–80). The mites were mounted in Heinze PVA medium, subsequently counted and identified microscopically. The results were calculated as the number of mites in a gram of dust. If no mites were found, the limit of detection was calculated for that sample [24–26].

Symptom questionnaire and nasal lavage

Twenty-eight kitchen workers participated in the study. A group of eight clerical personnel from the same schools formed a reference group. Nasal lavage (NAL) was performed at the end of the spring term. Before nasal lavage, all the subjects filled in a one-page questionnaire on their health during the preceding week. The questionnaire included questions on laryngeal, lower airways, nasal, eye, dermal and non-specific symptoms. In addition, information about the atopic status and other background factors was obtained from a larger questionnaire study that will be reported separately.

Nasal lavage. NAL was performed as described earlier [14] with instillation of 4.5 ml prewarmed Hank's balanced salt solution (HBSS) (Gibco, Paisley, UK) (37°C) into the nostril through a heat-softened catheter. The cartilaginous bridge of the nose was vibrated with a neonatal percussor (Neo-Cussor, General Physiotherapy, Inc., St.

Louis, MO, USA) and the fluid was refluxed three times. The procedure was then repeated on the opposite nostril. The sample was centrifuged (425g for 10 min), the cells were resuspended in 2 ml of the supernatant, and the rest of the cell-free supernatant was removed. The remaining cell suspension was incubated for 24 h at 37°C and then centrifuged (425g for 10 min). The supernatant and cells were frozen at –70°C.

Nitric oxide and cytokine analysis. Nitric oxide(NO) in the NAL supernatant was assayed by the Griess reaction as the stable NO oxidation product nitrite [27]. Cytokines were analyzed by using DuoSet human TNF- α , IL-4 and IL-6 enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems Europe, Abingdon, Oxon, UK).

Cytospin. Cyto-centrifuge preparations were made by using 100 μ l of resuspended cell suspension, in which the mucus was broken by 0.5% dithiothreitol/0.1% bovine serum albumin. The solution was centrifuged and the slides were stained with May-Grünwald-Giemsa staining [28] for the cell differential counts.

Ethics

All the studied individuals were volunteers. The Ethical Committee of the Kuopio University Hospital approved the study protocol.

Statistical analysis

Non-parametric statistical tests were used because normal distributions of the variables could not be obtained by transformations. The Mann-Whitney test and Kruskal-Wallis one-way analysis of variance test were used to compare differences in fungal and bacterial concentrations in air and surface samples. Multiple comparisons were performed using Dunn's post hoc test [29]. Spearman rank correlation was used to analyze fungal concentrations between the two growth media. The average concentrations of microbes were reported as geometric means (GM), because concentration distributions were closer to lognormal than to normal distributions. NAL data were analyzed using the Mann-Whitney test. The SPSS statistical package [30], version 10, was used for analyses.

RESULTS

Microbes in the indoor air

Total concentrations of viable airborne fungi on the two growth media correlated ($r_s = 0.81$, $p < 0.0001$) therefore, statistical comparisons of total counts were made using the combined data from both media. Fungal concentrations were lower ($p = 0.026$) in the kitchens (GM = 12.5 cfu/m³, range, ≤ 2 –650 cfu/m³) than in the other facilities of the same school buildings (GM = 19.1 cfu/m³, range, ≤ 2 –510 cfu/m³). Figure 1 illustrates the distributions of fungal concentrations in the moisture-damaged and reference schools. The fungal concentrations were

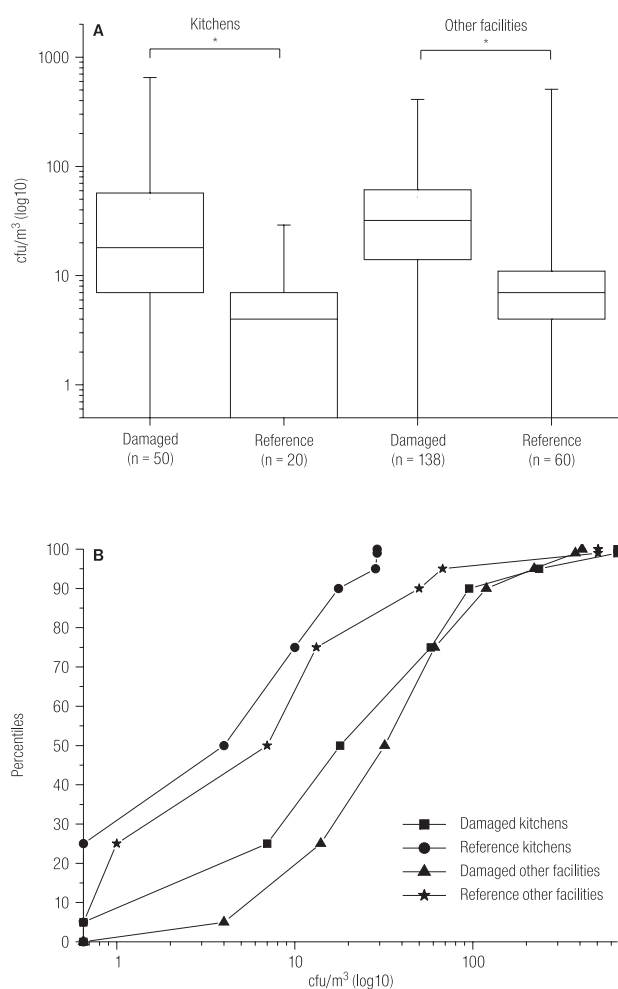


Fig. 1. Concentrations of viable airborne fungi in kitchens and in other facilities of damaged and reference schools. (A) Each box represents the interquartile range of values (25th – 75th percentile), median is indicated by a horizontal line. Whiskers show the minimum and maximum values. Asterisks indicate statistically significant difference ($p < 0.05$). (B) For further clarification of the concentration distributions, cumulative frequency distributions are also shown.

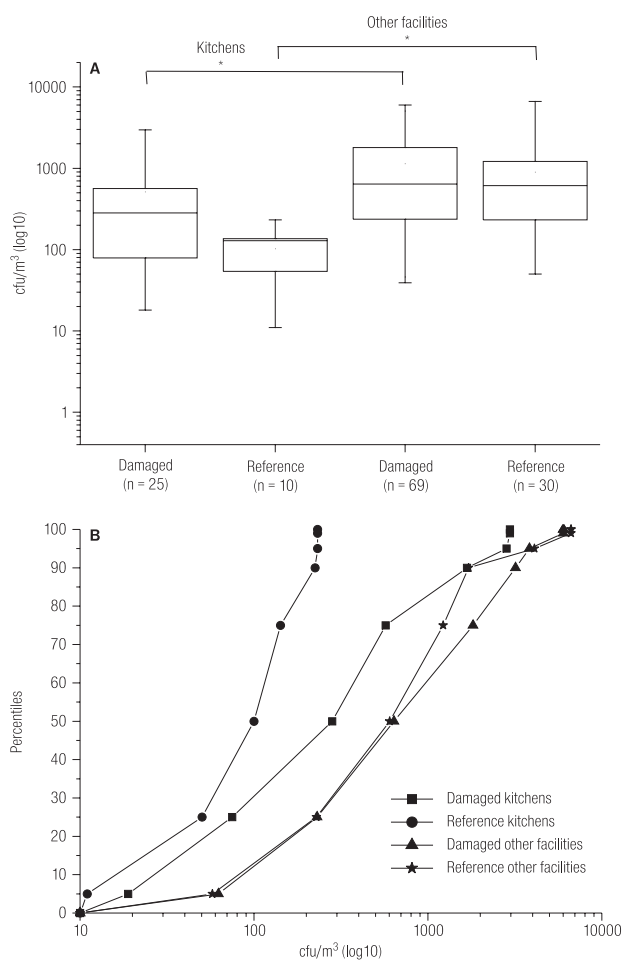


Fig. 2. Concentrations of viable airborne bacteria in kitchens and in other facilities of damaged and reference schools. (A) Each box represents the interquartile range of values (25th – 75th percentile), median is indicated by a horizontal line. Whiskers show the minimum and maximum values. Asterisks indicate statistically significant difference ($p < 0.05$). For further clarification of the concentration distributions, cumulative frequency distributions are also shown.

higher ($p < 0.05$) in the kitchens of the damaged schools than in those of the reference schools. Similarly, the concentrations in the other facilities of the schools were higher ($p < 0.05$) in the damaged than in the reference schools. Consistently with fungi, the concentrations of viable airborne bacteria were lower ($p < 0.001$) in the kitchens (GM = 160 cfu/m³, range, 11–3000 cfu/m³) than in other facilities of the schools (GM = 556 cfu/m³, range, 39–6700 cfu/m³). The difference between kitchens and other facilities remained significant ($p < 0.05$) even after classifying the schools into damaged and reference schools (Fig. 2). The difference in concentrations of bacteria between damaged and reference kitchens was not significant.

Table 1. Microbial biota found in indoor air samples of the moisture-damaged schools (A) and reference schools (B), and in surface samples of the kitchens

	Air samples				Surface samples		
	Kitchens		Other facilities		Damaged surfaces (n = 68)	Undamaged surfaces	
	A (n = 50)	B (n = 20)	A (n = 138)	B (n = 60)		Damaged kitchens (n = 122)	Reference kitchens (n = 20)
<i>Acremonium</i>	+		+		++	+	
<i>Aspergillus spp.</i>	+++		+++	++		+	++
<i>A. fumigatus</i>				+		+	
<i>A. niger</i>							++
<i>A. versicolor</i>	++	+	++	++	+	+	
<i>Aureobasidium</i>	++		++	+	++	++	++
<i>Basidiomycetes</i>		++					
<i>Botrytis</i>	+	+				+	
<i>Calcarisporium</i>			+				
<i>Chrysosporium</i>			+				
<i>Cladosporium</i>	+++	++	+++	++	++	+	
<i>Drechslera</i>					+		
<i>Eurotium</i>	+		++	+		+	
<i>Exophiala</i>					+	+	
<i>Fusarium</i>					+		
<i>Geomyces</i>	++		++	+		+	
<i>Gonatorrhodiella</i>				+			
<i>Hyalodendron</i>			+				
<i>Monocillium</i>			+		+		
<i>Mucor</i>		+	+				
Non-sporing isolates	+++	++	+++	+++	+++	++	+++
<i>Ochroconis</i>					++	+	
<i>Oidiodendron</i>	+		+				
<i>Olipitrichum</i>	++		+	+			
<i>Paecilomyces</i>			+			+	
<i>Penicillium</i>	+++	+++	+++	+++	++	++	+++
<i>Phialophora</i>						+	
<i>Phoma</i>							++
<i>Rhinoctadiella</i>	+		+				
<i>Scopulariopsis</i>			+				
<i>Sphaeropsidales-group</i>	+	+	+	++	+++	++	+
<i>Sporobolomyces</i>	+						
<i>Stachybotrys</i>			+				
<i>Trichoderma</i>	+		+				
<i>Tritirachium</i>	+		+				
<i>Ulocladium</i>			+	+	+		
Unknown	+		++	+	+	+	
<i>Wallemia</i>	++		++	+	+		
Yeasts	+++	+++	+++	+++	+++	+++	+++
Actinomyces	+++	++	+++	++	++	++	
Total number of genera, species or groups	21	10	28	17	17	19	8

Bolded are regarded as moisture damage indicators [31].

+ Found in 1–5% of samples; ++ Found in 6–24% of samples; +++ Found in ≥ 25% of samples.

The fungal diversity in the indoor air of kitchens and other school facilities is presented in Table 1. With minor exceptions, the most frequent fungi in all the facilities were *Penicillium*, *Cladosporium*, yeasts, non-sporing isolates and *Aspergillus*. In the two reference kitchens, basidiomycetes were frequently found, but they were not found in any other facilities. In the other facilities of the damaged schools, concentrations of *Penicillium*, *Cladosporium* and yeasts were higher ($p < 0.05$) on both media than those in reference schools. Furthermore, actinomycetes were more frequent ($p = 0.004$) in bacterial samples from the moisture-damaged schools.

Microbes on the surfaces

Concentrations of viable fungi on the two sampling media correlated ($r_s = 0.92$, $p < 0.0001$) therefore, statis-

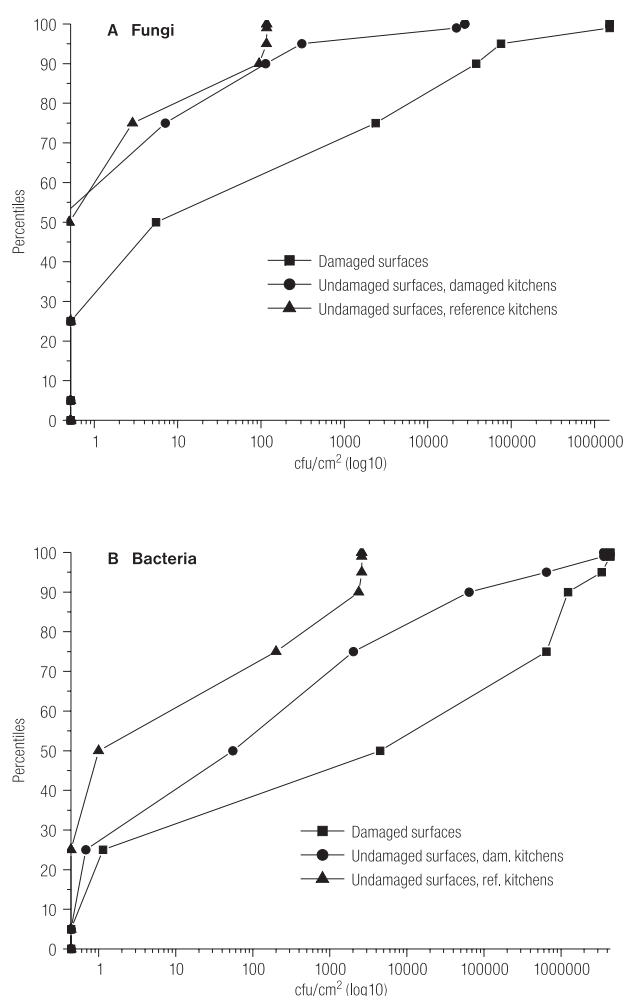


Fig. 3. Cumulative frequency distributions of fungal and bacterial concentrations on kitchen surfaces.

tical comparisons were again made by combining the data from both media. The cumulative distributions of concentrations of fungi on surfaces in kitchens of the moisture-damaged schools and reference schools are presented in Fig. 3A. Fungal concentrations were higher ($p < 0.001$) on damaged surfaces (GM = 51 cfu/cm², range, 0–1 500 000 cfu/cm²) than on undamaged surfaces (GM = 3 cfu/cm², range, 0–28 000 cfu/cm²). Although the concentrations remained under the limit of detection in almost one third of samples from damaged surfaces, concentrations 10³–10⁶ cfu/cm² were also detected. The distribution of concentrations had a similar pattern on both damaged and undamaged surfaces in moisture-damaged kitchens. On undamaged surfaces of reference kitchens, the concentrations remained below 200 cfu/cm². The diversity of fungi on surfaces is presented in Table 1. Yeasts were most frequent on all the surfaces. Concentrations of yeasts were higher ($p < 0.05$) on damaged than on undamaged surfaces of damaged kitchens (2% MEA only).

Figure 3B shows corresponding distributions for bacterial concentrations on surfaces. The concentrations of bacteria were higher ($p = 0.005$) on damaged sur-

Table 2. Comparison between the results of the Mycometer-test and culture method of surface samples

Sample	Mycometer*		Culture (cfu/cm ²)	
	Number	Category	M2	DG18
1	0	A	1	0
2	2	A	0	0
3	19	A	0	0
4	3	A	1	1
5	5	A	4	3
6	19	A	3	3
7	7	A	68	55
8	-2	A	153	152
9	125	B	284	158
10	213	B	0	439
11	42	B	952	997
12	150	B	4250	3750
13	177	B	15950	18300
14	457	C	70955	6495
15	2207	C	33455	9273

* Mycometer-number ≤ 25 . Category A = the level of mold is not above normal background level. $25 < \text{Mycometer-number} \leq 450$. Category B = the level of mold is above normal background level. Mycometer-number > 450 . Category C = the level of mold is high indicating mold growth.

faces (GM = 1715 cfu/cm²; range, 0–4 400 000 cfu/cm²) than on undamaged surfaces (GM = 67 cfu/cm²; range, 0–3 600 000 cfu/cm²). However, in half of the samples from undamaged surfaces bacterial concentrations in moisture-damaged kitchens were one or two orders of magnitude higher than in reference kitchens.

A comparison between the results of the Mycometer-test and cultivation method of surface sampling is presented in Table 2. With only a few exceptions, the interpretation of the results by both methods was consistent.

Other analyses

Most of the bacteria isolated from kitchen surfaces were gram-negative rods, and a quarter were gram-positive cocci, including *Micrococcus* spp. and a few *Bacillus* spp. (Fig. 4). On surfaces, gram-negative bacteria (GM = 499 cfu/cm²; range, 0–650000 cfu/cm²) and thermophilic bacteria (GM = 19 cfu/cm²; range, 0–4400 cfu/cm²) were detected. Mycobacteria were found in four surface samples (range, 1–25 cfu/cm²). In material samples, fungal concentrations ranged from < 45 to 540 000 cfu/g (GM = 224 cfu/g). In these samples, actinomycetes (GM = 232 cfu/g; range, 0–130 000 cfu/g) and other mesophilic bacteria (GM = 13 284 cfu/g; range, 1200–4 300 000 cfu/g) as well as thermophilic bacteria (GM = 68 cfu/g; range < 45–14 000 cfu/g) were found. Furthermore, in one material sample with the highest concentrations of actinomycetes and thermophilic bacteria, a relatively high number of mycobacteria ($4.0 \cdot 10^6$ cfu/g) were found.

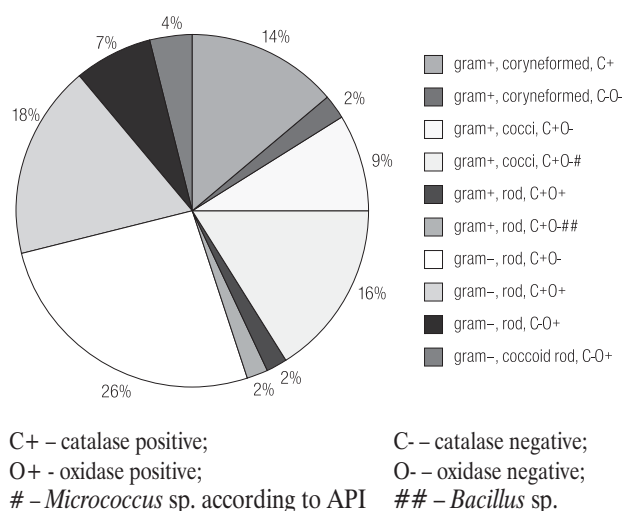


Fig. 4. Bacterial typing in the school kitchens.

One *Bacillus cereus*-strain and another *Bacillus*-typed catalase positive rod were isolated.

Of the 15 vacuumed samples analyzed, 33% contained storage mites (range, 0–60% per kitchen) and there were 20 mites in a gram of dust on average (range, 0–33.3 mites/g). Prominent genera were Mesostigmata, Acaridae, *Demodex* spp. and *Tyrophagus* spp.

Nasal lavage samples

Concentration of NO and cytokines in NAL fluid. Production of NO, assessed as nitrite levels in nasal lavage fluid, was slightly increased among kitchen workers (median, 0.8 μ M; range, 0.2–17 μ M) as compared to clerical personnel (median, 0.5 μ M; range, 0.2–1.1 μ M). The difference, however, was not statistically significant. Median concentration of IL-4 in the NAL fluid was higher among kitchen workers (25 pg/ml; range, 0–200 pg/ml) than among clerical personnel (0 pg/ml; range, 0–37 pg/ml) ($p = 0.059$). Production of IL-6 was slightly increased in the NAL samples of kitchen workers (4.8 pg/ml; range, 0–240 pg/ml), as compared to those of clerical personnel (3.2 pg/ml; range, 0–68 pg/ml) ($p = 0.588$). Also, the highest TNF- α values were detected in kitchen workers (Fig. 5). In the cell differential count, neutrophilic cells dominated NAL cell profiles. Few samples contained occasional macrophages, eosinophils, and lymphocytes. There were no differences in cell differential counts between kitchen and clerical personnel.

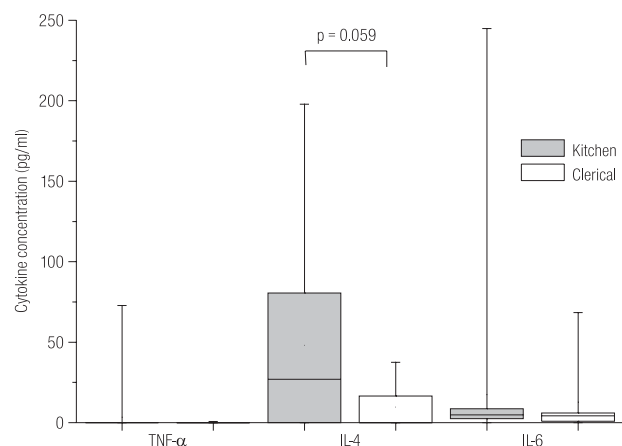


Fig. 5. Concentrations of TNF- α , IL-4 and IL-6 in the NAL samples of kitchen and clerical personnel. Each box represents the interquartile range of values (25th–75th percentile), median is indicated by a horizontal line. Whiskers show the minimum and maximum values.

Table 3. Symptoms reported by the kitchen and clerical personnel

	Symptoms					
	Lower airways n (%)	Laryngeal n (%)	Nasal n (%)	Eye n (%)	Skin n (%)	Nonspe- cific n (%)
Kitchen (n = 28)	15 (54)	15 (54)	23 (82)	8 (29)	2 (7)	20 (71)
Clerks (n = 8)	6 (75)	4 (50)	7 (88)	3 (38)	2 (25)	7 (88)

Questionnaire on health status. The prevalence of respiratory and general symptoms was high among both kitchen and clerical personnel. Of the 13 persons with high IL-4 concentration, 11 (including 9 kitchen personnel), had reported allergies and repeated respiratory infections. Atopic diseases were present in 42% of kitchen personnel. There were no statistically significant differences in the reporting of symptoms between kitchen workers and clerical personnel (Table 3).

DISCUSSION

Microbial conditions in institutional kitchens were assessed. The effect of moisture damage on the microbial status of school facilities was studied by comparing six moisture-damaged schools with two reference schools. Symptoms in kitchen and clerical personnel were surveyed and inflammatory mediators in nasal lavage fluid analyzed.

Fungal concentrations in the indoor air of kitchens were lower than in the other facilities of the schools (GM = 12.5 cfu/m³; range, ≤ 2–650 cfu/m³ vs. GM = 19.1 cfu/m³; range ≤ 2–510 cfu/m³). This may be explained by more effective ventilation in the kitchens than in the other parts of the buildings. Furthermore, other parts of the schools, such as classrooms and corridors are more crowded than kitchens, and thus the resuspension of spores from floors and their drifting with clothes may explain the difference [32]. In general, our findings on fungal concentrations in other school facilities were similar to those reported earlier in the northern climate [33,34]. The results from other facilities in these buildings have been reported in detail as a part of a larger school study [35]. However, also this sample of schools showed the increasing effect of moisture

damage on fungal concentrations both in kitchens and in other facilities of schools.

Studies dealing with microbial contents of indoor air in kitchen types of environments are scant. Marchant et al. [9] found concentrations similar to our results in a kitchen of a hospital. In a cafeteria [36], however, the range of airborne moulds (0–4565 cfu/m³) was wider than in our study. The high concentrations were probably explained by the time of sampling, since the highest concentrations were found in the morning during vegetable washing and peeling, but they declined in the afternoon. Handling of vegetables has been shown to be a significant source of indoor microbes [32]. Low fungal concentrations in our study may also be explained by humid conditions in kitchens. Due to massive moisture load during washing and cooking, relative humidity of air (RH) remains high for long time. As reported earlier [17], RH values in kitchens may exceed 80%, an unusually high humidity in the cold winter climate, and in those moist conditions, less fungal spores may be released into the air [37].

Similarly to fungi airborne bacterial concentrations were significantly lower in the kitchens than in the other facilities of the schools (GM = 160 cfu/m³; range, 11–3000 cfu/m³ vs. GM = 556 cfu/m³; range, 39–6700 cfu/m³). This is evidently due to the lower occupancy in kitchens and their more effective ventilation. There are probably two major sources of bacteria in kitchens: humans, a well-known source of indoor air bacteria [38], and activities typical of kitchens such as handling of vegetables and washing, as shown by Wójcik-Stopczyńska et al. [36] in a cafeteria with bacterial concentrations of 75–4550 cfu/m³. Unlike fungal concentrations, the effect of moisture damage on bacterial concentrations was not significant in our study.

With only minor exceptions, the composition of airborne mycobiota was similar in the kitchens and the other facilities of the schools. The most common genera found in kitchen were similar to those generally found in indoor environments [33,39,40]. The effect of moisture damage was specifically seen as elevated levels of *Penicillium*, *Cladosporium* and yeasts in the other facilities of the schools. Similar effect on *Cladosporium* has been reported from residences [39].

The concentrations of fungi were significantly higher on moisture-damaged than on undamaged surfaces

(GM = 51 cfu/cm²; range, 0–1 500 000 cfu/cm² vs. GM = 3 cfu/cm²; range, 0–28 000 cfu/cm²). Fungal concentrations of 10⁶ cfu/cm² on damaged surfaces are typical levels detected on visibly damaged sites [41]. Fungal growth was also detected with the Mycometer-test. Furthermore, microbial levels in damaged kitchens proved to be higher even on undamaged surfaces, even though the difference did not reach statistical significance. Thus, undamaged surfaces also show contamination from moisture-damaged sites. The predominant fungi on all kitchen surfaces were yeasts, like in an apartment kitchen [42].

Bacterial, like fungal, concentrations were also significantly higher on moisture-damaged than on undamaged surfaces (GM = 1715 cfu/cm²; range, 0–4.4 • 10⁶ cfu/cm² vs. GM = 67 cfu/cm²; range, 0–3.6 • 10⁶ cfu/cm²). Bacterial concentrations of 10²–10⁵ cfu/cm² have been detected on surfaces such as sink basins, faucet handles and tables in domestic kitchens [43], but targeted disinfectant use decreased the concentrations to 1–10² cfu/cm². Mattick et al. [44] have pointed out that bacterial concentrations in institutional kitchens may be lower than those in domestic kitchens due to higher water temperature, use of industrial detergents and staff training in food hygiene. On the other hand, surfaces in contact with products and food are usually cleaned several times per day, while environmental surfaces such as walls are cleaned less frequently. Thus, our results represent the background concentrations of bacteria in kitchens.

The majority of isolated bacteria were gram-negative rods. The bacterial contamination of moist surfaces of domestic kitchens has been shown to be mostly due to gram-negative rods, like in our study, and coliforming bacteria [12,13,42]. In our study, a quarter of the isolated bacteria were gram-positive cocci, many of them are present in the normal flora in humans [45]. Mycobacteria were found in four of our surface samples and in one of the material samples in the concentration similar to that observed in corresponding materials [46]. In the latter, also *Bacillus cereus* was found as well as large numbers of other bacteria.

The overall mite density was low and below the suggested threshold value for house dust mites [47]. The mites found in the kitchens were storage mite species, for which no

threshold values have been suggested, although they may cause allergic symptoms and sensitization [7]. Seasonal variation can explain the low mite density detected in winter; the density tends to grow in summer and it is highest in autumn [48]. The occurrence of storage mites in indoor environments other than actual storage facilities has been found earlier [47–49].

The prevalence of respiratory and general symptoms was high among kitchen and clerical personnel. The majority of respondents (86%) worked in the moisture-damaged schools, and thus, the high symptom prevalence is evidently associated with the exposure occurring in damaged environments. The proportion of atopic individuals among kitchen workers was rather high (42%) in relation to the general population [50]. The concentrations of measured cytokines in NAL samples tended to be higher among kitchen workers than among clerical personnel. The production of IL-4 was further associated with self-reported allergies and repeated respiratory infections. This finding suggests some work-related exposure capable of inducing inflammatory responses in the upper airways and affecting the health status of the personnel. Although causative exposure agents are still obscure, an association between increased cytokine levels in NAL and exposure in moisture-damaged school building has been shown in previous studies [14,15]. In a recent study, the increased IL-4 concentration in NAL fluid seemed to be associated particularly with bacterial exposure [51].

In summary, airborne microbial concentrations in kitchens were lower than in other facilities of the schools. However, a more thorough assessment of microbial status in kitchens by means of surface samples showed that microbial exposure in kitchens is possible. In the health survey, the high prevalence of symptoms and increased concentrations of NAL cytokines in kitchen personnel were found.

Moreover, the occurrence of moisture damage increased microbial concentrations in indoor air as well as on surfaces and structures of kitchens. Thus, kitchen facilities differ from other facilities of the school building for their moisture conditions and microbial contamination. They represent a specific type of environment that may affect the occupational health of the personnel.

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