Moulds, bacteria and MVOC in classroom and outdoor air, and microbial components in settled dust from schools in Shanghai, China

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ABSTRACT
Thirty classrooms in 10 schools in Shanghai, China, were investigated in winter. Dust was collected by vacuum cleaning, analysed for ergosterol, muramic acid, and 3-hydroxy fatty acids (LPS) by tandem mass spectrometry (GC-MSMS). Airborne microoganisms were sampled on Nucleopore filters (CAMNEA). The compound 1-octen-3-ol was found in higher concentrations in indoor than in outdoor air. Total indoor bacteria were positively correlated to both LPS and muramic acid in settled dust. Indoor and outdoor air contained many viable species. Viable indoor moulds were positively correlated to 1-octen-3-ol in the air, and ergosterol in dust. In total, 5.4% of the pupils ($N = 1414$) reported mould allergy/intolerance, and these complaints were higher in one school with building dampness. In conclusion, we found a relationship between microbial components in settled dust and in classroom air, and some types of mould were found only in indoor air samples,, but total indoor and outdoor concentrations were similar.

INDEX TERMS
Allergy; Mould; Bacteria; Endotoxin; Schools

INTRODUCTION
There are few international publications on the school environment from Asia, or China (Lee *et al*., 2000; Su *et al.*, 2001). Building dampness and moulds may increase the risk for asthma and allergies (e.g. Bornehag *et al.*, 2001). There are few health studies on microbial exposure in schools (Smedje *et al.*, 1997; Su *et al.*, 2001). Different approaches can be applied when monitoring indoor microbial components. Measurements of airborne microorganisms, and volatile organic compounds of possible microbial origin (MVOC), have been performed. Some researchers have analysed microbial components in settled dust. The main aim was to measure and compare airborne microorganisms and MVOC in indoor/outdoor air of schools in Shanghai, and different microbial components in settled dust from classrooms. In addition, we compared microbial exposure, current asthma, and reports on mould allergy/intolerance in schools with and without signs of building dampness.

METHODS
Study Design
In China, there is a 6-year primary school, followed by a 3-year junior high school. Shanghai is the largest city in China with approximately 16 million inhabitants, situated at the coast in mid-China. The main aim of the project was to form a cohort of pupils, and follow their development of asthma and allergies during a 2-year period, when the classes remained in the same schools. The school administration in two people's communes in Shanghai were contacted. One commune was situated in central Shanghai, with a total of 30 primary schools.

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The other was in the western part of Shanghai near the major river, with a total of 24 primary schools. Five schools were randomly selected from each of the two communes. The headmasters were contacted, and all schools agreed to participate.

Questionnaire Study

In Shanghai, three classes from the 1st form were selected from each of the 10 schools. All pupils in the 30 classes ($N = 1435$) received a questionnaire in November 2000. It included the core questionnaire from the International Study Group of Asthma and Allergies in Childhood (ISAAC, 1998). It also contained additional questions from the previous Swedish school study (Smedje *et al.*, 1997), and some questions from the European Community Respiratory Health Survey (ECRHS) (Janson *et al.*, 2001). The questions were translated from Swedish or English to Chinese by one person, and back-translated by another person. The questionnaires were distributed by the teachers, and were answered by the pupils the same day. Totally, 1414 pupils participated (99%). This presentation deals only with self-reported mould allergy, cumulative prevalence of asthma and current asthma. Current asthma was defined as having either current asthma medication, or having had an asthma attack during the latest 12 months. Detailed data from the questionnaire study will be presented elsewhere.

Building Inspection and Microbial Measurements

Measurements of microorganisms, and microbial components were performed in November–December 2000. Measurements were performed in the home-classrooms ($N = 30$) where the pupils spend most of the time. The buildings and classrooms were inspected, and details on constructions, materials, type of ventilation system and signs of building dampness were noted. Airborne microorganisms and VOC of possible microbial origin (MVOC) were sampled simultaneously, in the classrooms (three samples per school) and outside the building (one sample per school). Airborne microorganisms were sampled on 25 mm Nucleopore filters (pore size 0.4 µm) (2.0 l/min; 4 h). The total concentration of airborne moulds and bacteria was determined by the CAMNEA method (Palmgren *et al.*, 1986). Viable moulds and bacteria were determined by incubation on two different media. The detection limit for viable organisms was 30 colony forming units (cfu) per m^3 of air. Airborne MVOC were sampled on a charcoal tube (Anasorb 747, SKC Inc) (0.25 l/min; 4 h). The tubes were desorbed with 2 ml of methylene chloride, and analysed by selective ion monitoring (SIM) gas chromatography mass spectrometry GCMS (Wessen and Shoeps, 1995). The following compounds were measured; 3-methylfuran, 1-butanol, 2-butanol, 2-pentanol, dimethyldisulfid (DMDS), 2-hexanone, 2-heptanone, 1-octen-3-ol and 3-octanone. The total concentration of the selected MVOC was calculated, by mass summation, excluding the butanols.

Settled dust was collected with a vacuum cleaner fitted with a special dust collector (ALK Abello, Copenhagen, Denmark) equipped with a Millipore filter (pose size 6 µm). Vacuum cleaning was performed over desks, chairs, shelves and floor over a period of 4 min per sample, 2 min on the floor and 2 min on other surfaces. Three classrooms were divided into two halves (corridor half and window half), and two samples per classroom were taken. The samples from the window half were analysed for microbial components. The filters were sealed in plastic bags and stored at –20°C until extraction. The dust samples (1–2.3 mg aliquots) were hydrolysed and further processed for analysis of the content of 3-hydroxy-fatty acids with 10–18 carbon atoms, muramic acid, and ergosterol, by tandem GCMS (Saraf *et al.*, 1999). In brief, 3-hydroxy fatty acids were analysed as methyl ester/trimethylsilyl (TMS) derivatives after strong acid methanolysis of dust samples, extraction and silica gel column purification. Ergosterol was analysed as a TMS derivative, after saponification of dust samples, followed by extractions and purification using silica gel column. Muramic acid was analysed after acid methanolysis of samples and removal of lipid material. The 3-hydroxy fatty acids are markers of the lipopolysacharides (LPS) in the outer membrane of Gram-negative bacteria (endotoxin). Muramic acid is marker of peptidoglycan, a basic constituent of eubacterial cell walls. Ergosterol is a marker of fungi.

RESULTS

The mean age of the school buildings was 33 years (range 3–65 years). None had a mechanical ventilation system, air-conditioning or any heating system. All were concrete buildings with openable windows. One classroom (3%) had a wooden floor. The other classrooms (97%) did not have any floor covering, the floor consisted of unpainted concrete. One school in central Shanghai (school 4) had signs of building dampness, with water leakage and visible moulds. All schools had daily floor cleaning and daily desks cleaning. There were no significant difference between indoor and outdoor concentrations of either viable or total bacteria, or viable or total moulds (Table 1). The school with signs of building dampness had the highest average indoor concentration of total moulds (Table 1), and the highest concentration of muramic acid in dust (Table 2). When comparing schools without any signs of building dampness in both communes (excluding school 4), the classrooms in central Shanghai had higher indoor concentrations of 1-octen-3-ol (69 versus 53 ng/m^3) ($p = 0.05$), muramic acid (37 versus 17 ng/mg dust), and LPS (49 versus 23 pmol/mg dust). There were no significant differences between the two communes for viable or total moulds or bacteria, 3-methylfuran, total MVOC, ergosterol, or total amount of settled dust.

Both indoor and outdoor air contained many different species of viable moulds and bacteria. The outdoor air in the central commune contained *Acremonium*, *Alternaria*, *Aspergillus spp*, *Aspergillus niger*, *Bacillus*, *Cladosporium*, *Dematiaceous hyhomycetes*, *Penicillus* spp, *Pseudomonas*, *Trichoderma* and *Yeast*. The outdoor air in the western commune contained *Aurobasidius*, *Aspergillus* spp, *Aspergillus versicolor*, *Aspergillus ochraeus*, *Bacillus, Cladosporium*, *Penicillus* spp, *Pseudomonas*, *Paecilomyces*, *Phoma*, *Streptomyces*, and *Yeast*. The indoor air samples contained some 'indoor species', not detected in any of the outdoor air samples. The school with building dampness contained *Eurotium*. The other four schools from the central commune contained *Aspergillus penicilloides*, *Eurotium*, *Rhizopus*, *Sporothrix*, *Ulocladium*, and *Wallemia*. The five schools from the western commune contained *Aspergillus penicilloides*, *Aspergillus flavus*, *Aspergillus niger*, and *Eurotium*. The majority (80%) of both indoor and outdoor air samples contained *Pseudomonas*, the only Gram-negative bacteria that was identified by the laboratory.

Table 1 Mean indoor/outdoor concentration of airborne microorganisms

	Year of construction	Viable bacteria I/O	Total bacteria I/O	Viable moulds I/O	Total moulds I/O
Central Shanghai					
School 1	1964	610/790	10 500/12 000	880/790	27 700/24 000
School 2	1964	7800/8900	109 000/130 000	2900/1600	11 700/12 000
School 3	1935+1974	1800/2400	76 000/23 000	830/670	32 000/35 000
School 4 (damp building)	1958	5900/1000	20 000/6000	1700/880	72 300/35 000
School 5	1957	680/40	8200/<1300	830/110	10 200/<1300
Western Shanghai					
School 6	1963	1180/660	14 300/92 000	750/440	12 800/5500

School 7	1988	5600/3600	424 000/120 000	890/2300	9700/6000
School 8	1963+1997	1600/23 000	5500/270 000	680/2300	5500/83 000
School 9 (rain)	1978	5200/55	8000/5500	1930/220	8000/550
School 10	1964	1440/16 000	80 300/80 000	180/650	7300/80 000
Mean	1967	3200/5600	78 000/74 000	1180/820	20 200/34 600

I = indoor concentration per m^3 (mean of three samples); O = outdoor concentration per m^3 (one sample).

Table 2 Mean concentration of MVOC and microbial components in dust from classrooms

	Total dust (mg)	LPS (pmol/ mg)	Muramic acid (ng/mg)	Ergostero l (ng/g)	1-Octen-3-ol (ng/m^3)	3-Methyl furan (ng/m^3)	MVOC ($\mu g/m^3$)
Central Shanghai							
The damp school (4)	98	23	62	5.5	83	40	0.30
Schools 1, 2, 3, 5	86	37	49	7.7	69	38	0.28
Western Shanghai							
Schools 6–10	134	17	23	6.2	54	100	0.26
All classrooms (N = 30)	116	26	37	6.8	63	62	0.27
(Min–Max)	(16–352)	(8–111)	(8–95)	(0.6–30)	(23–130)	(21–440)	(0.14–0.52)

Table 3 Correlation between microbial components in classroom air and settled dust

Settled dust pollutant	Airborne microorganisms	Correlation coefficient	Two-tailed P value
Total amount of dust per sample	Total bacteria	0.56	0.003
Concentration of LPS	Total moulds	0.44	0.02
Amount of LPS	Total bacteria	0.37	0.05
Concentration of muramic acid	Total moulds	0.55	0.004
Amount of muramic acid	Total bacteria	0.41	0.04
Amount of muramic acid	Total moulds	0.41	0.04
Concentration of ergosterol	Viable moulds	0.47	0.02
Amount of ergosterol	Total bacteria	0.44	0.02

Amount = concentration of microbial component in dust × total amount of dust.

When comparing indoor and outdoor levels of MVOC, concentration of 1-octen-3-ol was higher indoors ($p < 0.01$), but there were no significant differences for 3-methylfuran or total MVOC. The mean outdoor level was 38 ng/m^3 (range 13–82) of 1-octen-3-ol, 52 ng/m^3

(range 32–110) of 3-methylfuran and 0.21 μg/m^3 (range 0.13–0.44) for total MVOC (excluding butanols). As a next step, correlation between microorganisms and MVOC in classroom air, and microbial components in settled dust were calculated (Table 3). Finally, prevalence of hay fever (pollinosis), asthma and reports on mould allergy/intolerance were compared between the schools. Pupils in the damp school building reported significantly more mould allergy/intolerance, as compared to the other nine schools ($p < 0.001$), and current asthma was twice as common in the damp school. When comparing schools without any of building dampness in both communes (excluding school 4), there were no significant differences in hay fever, mould allergy/intolerance, asthma, or asthma medication (Table 4).

Table 4 Hay fever, asthma and mould allergy/intolerance among pupils in Shanghai

	Central Shanghai		Western Shanghai	Total prevalence	*P* value
	Damp	No damp			
Hay fever (%)	5.7	3.9	4.5	4.4	NS
Mould allergy/intolerance (%)	11.3	6.5	3.4	5.5	<0.001
Ever had asthma (%)	11.3	8.5	11.5	10.3	NS
Asthma medication (%)	2.8	1.8	2.6	2.3	NS
Current asthma attacks (%)	5.0	1.9	2.3	2.4	NS
Current asthma (%)	5.0	2.3	3.4	3.1	NS

[a]Comparison between the school with building dampness, and all other schools (Chi-square).

DISCUSSION

Most health investigations of indoor microorganisms have been performed in temperate climate in Europe or North America (see Bornehag *et al.*, 2001). The cold and dry climate limits the natural growth of microorganisms, particularly outdoors. In the cold climate, there is a large temperature difference between the outdoor and indoor surface of the building, and a thick and complex wall construction is used, with both thermal insulation and a plastic dampness barrier. Moreover, organic materials (e.g. wood, paper and synthetic polymers) are commonly used, and the majority of the workplace buildings has a mechanical ventilation systems. Shanghai is the most wealthy part of China, situated in a subtropical coastal area. The outdoor daily mean temperature during the sampling period (November–December) was 8–17°C, and the outdoor air humidity was 40–97% RH. Somewhat higher temperatures and humidity levels were measured in the classrooms. All buildings had a simple wall construction, made of bricks or concrete, and no heating or mechanical ventilation system was installed.

We found 2–10 times higher indoor levels of moulds and bacteria in the Shanghai schools, as compared to randomly selected schools (Smedje *et al.*,1997) and dwellings in central Sweden (Norbäck *et al.*,1995) applying the same CAMNEA method. These differences are most likely due to higher outdoor levels of microorganisms in Shanghai. The majority (80%) of both indoor and outdoor air samples contained *Pseudomonas* sp., a Gram-negative bacteria containing endotoxin (LPS), which is suggested to be protective against allergy development.

A higher outdoor level of microorganisms and MVOC is probably a natural condition in the tropical or a sub-tropical warm climate in many parts of Asia, and the indoor concentration is strongly influenced by frequent window opening. In contrast, the buildings in a temperate climate, with little window opening and mechanical ventilation systems, creates a separate indoor environment. Relatively high levels of indoor and outdoor levels of viable moulds have been reported from Taiwan schools (Su *et al.*, 2001), but due to methodological differences, their data are not directly comparable. The Shanghai schools contained little organic building material, reducing the possibility for indoor microbial growth, despite high air humidity. Visible building dampness was found in 10% of the classrooms, a similar figure as in central Swedish junior high schools, where 4 out of 28 classrooms (14%) had visible signs of building dampness (Smedje *et al.*, 1997). The increase of reports on mould allergy/intolerance and current asthma in the damp school agrees with previous data, suggesting a two to three times increase of asthma and asthmatic symptoms (Bornehag *et al.*, 2001), as well as clinically verified Ig-E mediated mould allergy in damp buildings (Norbäck *et al.*, 1999).

CONCLUSIONS AND IMPLICATIONS

Concentrations of moulds, bacteria and MVOC were similar indoors and outdoors, suggesting that the indoor concentration of microbial pollutants are to a large extent determined by the outdoor concentration. The observed relationship between microbial components in settled dust, and in the classroom air, suggests that microbial compounds in settled dust can be used as an exposure indicator. Finally, building dampness and mould growth in schools in a subtropical area may increase the risk for allergy or hypersensitivity problems among pupils.

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