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Characterization of Bacteria and Fungi Bioaerosol in the Indoor Air of Selected Primary Schools in Malaysia

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Key Words
Bacteria and fungi bioaerosol · Indoor air · Primary Schools in Malaysia

Abstract
This study reports the types and concentrations of bacterial and fungal bioaerosols found in five randomly selected primary schools in Malaysia. Normal flora bacteria was the most frequently isolated bacteria including *Staphylococcus* spp., *Pseudomonas* spp. and *Bacillus* spp. *Terribacillus* spp. found in this study had never been reported before. The most frequently isolated fungal genera were *Aspergillus*, *Penicillium*, *Fusarium*, *Rhizopus* and *Zygomycetes*. The average concentration of bacteria in indoor and outdoor air were 1025 ± 612 CFU/m^3 and 1473 ± 1261 CFU/m^3, respectively, while the average concentration of fungal bioaerosol in indoor and outdoor air were 292 ± 83 CFU/m^3 and 401 ± 235 CFU/m^3, respectively. The percentages of bacterial and fungal samples that were within the American Conference of Industrial Hygenists (ACGIH) recommended levels were 44% and 33.8%, respectively. The ratio of indoor to outdoor fungi concentration was below 1.0, suggesting minimal indoor generative source for fungal bioaerosols. However, the ratio of indoor to outdoor bacteria concentration was approaching 1.0, suggesting the presence of potential internal generative source and inadequate ventilation. Building occupants might be one of the potential sources of bacteria in the indoor air as the bacteria concentrations without occupants were significantly lower than with occupants ($p < 0.05$).

Introduction

Bacterial and fungal bioaerosols are ubiquitous microorganism in the indoor environment and some can act as human airborne pathogens [1–3]. Normal flora rarely causes human illnesses although some are agents of hypersensitivity, infectious or inflammatory diseases. Endotoxin (a component of the outer membrane of Gram-negative bacteria) can inflame airways, elicit asthmatic attacks and cause bronchial hyper-reactivity [4]. *Cladosporium*, *Alternaria*, *Aspergillus* and *Fusarium* are

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among the most common fungal genera associated with symptoms of respiratory tract allergies [5].

Respiratory illnesses due to exposure of airborne contaminants are a common problem for humans, particularly for children. Mould allergy was found to be more frequent in children than in adults, although the causes of mould allergies are still uncertain [6]. According to Bayer et al., allergic diseases including nasal allergy, asthma and other allergies accounted for 20% loss of school days in both elementary and high schools in the US [7]. Exposure to these indoor air contaminants particularly among school children needs close attention as children are more susceptible to the infection or respiratory problems and a large portion of their time is spent in school.

The important sources of bioaerosols in residential and school environments are from humans and from outdoor air supply [8]. The risk of illness from environmental bacteria increases when the pupils enter building in inappropriate numbers [8,9]. Meklin et al. [10] found that high concentrations of viable airborne bacteria usually indicate insufficient ventilation of the building. In addition, epidemiological investigations have shown that sick building syndrome and hypersensitivity diseases are associated with exposure to large concentrations of airborne microbes [6,11].

Despite this, the information available on the types of airborne microbes and the exposure in buildings in tropical climates are currently limited, as most studies have been from temperate European regions with relatively few from the tropical Asian region. Findings on ventilation, microbial exposure and indoor air quality (IAQ) variables reported in cold climates [2,12–15] may not be applicable to tropical countries such as Malaysia, which experience little variation in temperature, humidity and rainfall throughout the year. A study on the IAQ of Child Care Centres (CCC) in tropical Singapore reported a significant difference between temperature and relative humidity compared with those in the cold countries [16]. Relative humidity, temperature and seasons showed positive correlations with concentration of microorganisms in indoor air [6,11,17,18].

This study aimed to characterize the type of bacterial and fungal bioaerosol that could be potentially pathogenic in the indoor air of primary schools using both phenotypic and genotypic identification as well as quantifying the concentrations. The findings of this study will help to establish standards for future reference. In addition, the study also sought to determine the correlation between airborne bacteria and fungi concentrations with temperature and relative humidity.

Materials and methods

Study design and location

This was a cross-sectional study carried out in five randomly selected primary schools in Hulu Langat from the list of schools obtained from the District Education Office. Hulu Langat is one of the largest districts in Selangor, Malaysia, with a mixture of urban and rural settlements. All selected schools were built more than 15 years ago, constructed of concrete, with no air-conditioning; the primary source of building ventilation would be via open windows and doors which could varied according to the size of the classroom and number of pupils in each classroom. Most of the schools have one teaching block with three floors to accommodate the classrooms, library, staff room, washroom and administrative office. The school canteen was located next to the teaching block. Prior to the environmental sampling, a 'walk-through' assessment was carried out to determine indoor and outdoor sampling locations. The indoor concentrations of bacteria and fungi measured in the schools were compared with the recommended guideline level [19,20] for an evaluation of the relative risk of exposure for the school children.

Sampling

The monitoring of bacterial and fungal bioaerosols, relative humidity and temperature were carried out during a 6-month period from November 2008 to June 2009. The indoor sampling of bioaerosol was conducted in the middle of classrooms for indoor air measurement and outside the selected classrooms (in the corridor) for outdoor air measurement. Sampling was also conducted in the school canteen beside the classroom. The school children spent about 38% of their school time in the school canteen for morning tea and lunch, waiting for their parents or the school bus to pick them up after school time, and for some teaching activities outside classroom. Samples were collected in the middle of the week during school days between the hours of 08:00 and 14:00. In each primary school, three classrooms, the classroom corridors and the canteen were randomly selected as the sampling points. Measurements were conducted at these locations as school children spent most of their school time at these locations. The children could suffer adversely due to the increase probability of exposure to potential pathogenic organisms at each of these locations, where bacterial and fungal bioaerosols, temperature and relative humidity were monitored simultaneously both indoors, in the middle of the classroom at the height of about 1.0 m (child's breathing zone), and outdoors at the corridor of selected classrooms. Sampling was carried out in duplicate at each of the measuring points.

The sampling device used for monitoring bacterial and fungal bioaerosols was the Duo SAS Super 360 microbiological air sampler (International P.B.I. S.p.A., Via Novara, 89 20153 Milano, Italia). The sampled air is drawn into the sampler through a plate perforated with 400 holes and laminar air flow is directed onto the agar surface of a contact plate on a standard 90 mm Petri dish for microbiological examination. The sampling time was 2 minutes, which corresponds to 200 litres of air as recommended by the manual produced by the manufacturer (International Pbi S.p.a., 2003). Before sampling, the inside of the sampler was disinfected with 70% alcohol and was then inserted with a media plate. Two Petri plates were placed inside the sampler at each sampling period: tryptic soy agar (TSA) for bacterial isolation, with cycloheximide 500 mg to suppress the growth of fungi and sabaroud dextrose agar (SDA) for fungal isolation with cycloheximide 100 mg to suppress the growth of bacteria. After the sampling was completed, plates were removed and incubated aerobically at 37°C for 1–2 days for bacteria culture and at 20–25°C for 3–5 days for fungi culture [21].

Bacterial and fungal colonies on agar plates were counted as colony forming units (CFU). Before calculating the concentrations as CFU/m^3 (see Eq 1) [22] they were corrected for the statistical possibility of multiple particles passing through the same hole using a 'positive hole correction table'.

$$\frac{X = \mathrm{Pr} \times 1000 \text{ CFU per 1000 litre of air } \left(1000 \text{ litre} = 1 \text{ m}^3\right)}{V} \tag{1}$$

where V is the volume of sampled air (200 litres of air), Pr is the probable count obtained by positive hole correction and X is the CFU per 1000 litre (1 m^3) of air.

Identification of Bacterial and Fungal Bioaerosol

Identification of bacterial bioaerosol was performed using both conventional and molecular techniques of laboratory diagnosis. Extensive phenotypic examination on pure culture including colony morphology examination, Gram-stain, oxidase and catalase activities, and biochemical profile, were done prior to genotypic identification using 16S RNA gene for confirmation. Bacterial isolates that showed Gram-positive cocci in cluster were cultured on Mannitol Salt Agar (MSA) and these were further identified using commercial identification strips that contain a series of biochemical test for *Staphylococcus* spp. (Microgen^{TM} Staphylococcus ID MID-69). Gram-positive bacilli were further analysed using the biochemical test kits GNA and GNB (Microgen, UK). Octal code was calculated based on positive test result and analysed using Microgen^{TM} Identification System Software.

Genotypic examination was performed using polymerase chain reaction (PCR) for 16S RNA. Sequencing of the 16S RNA gene is a universal bacterial identification method and has been used by bacterial taxonomists for a number of years as a measure of DNA similarity between isolates [23]. Bacterial DNA was extracted from pure bacteria culture grown overnight in Luria–Bertani (LB) broth according to the methods provided by GF-1 Bacterial DNA extraction kit (Vivantis Technologies Sdn. Bhd., Malaysia) user's guide protocol. Extracted DNA was amplified using PCR and the universal 16S RNA primers (Table 1). The composition of PCR mixture for the total volume of 25 µl reactions were 1 µl of forward and reverse primers (10 pmol), 17.0 µl of sterile distilled water, 5 µl of Mastermix (dNTPs, Taq polymerase, $MgCl_2$, PCR buffer, loading dye) provided by iDNA and 1 µl of template. Conditions consisted of 20 cycles each at 96°C (1 min), 57°C (1 min), 72°C (4 min) and cooling down at 4°C. All amplifications were performed in the thermalcycler (Biometra® T-Personal Thermocycler Biometra®, Gottingen, Germany). The amplification products were purified using GF PCR Purification Kit, run on gel electrophoresis and sent for commercial sequencing. 16S RNA sequencing results were compared with those available in the GenBank using the BLAST program through the National Center for Biotechnology Information (NCBI) server [24].

Fungal bioaerosols were identified at genus level based on micro- and macro-morphologies and reverse and surface colouration of colonies as grown on malt extract agar (MEA) and potato dextrose agar (PDA). Determination of the morphological structure of fungi was carried out by preparing a tease mount and cellophane tape mount in a modified mounting medium, lactophenol cotton blue [25].

Table 1. Primers nucleotide sequence use for PCR amplification of 16s RNA

Primers	Sequence	Reference
16S RNA Forward	5′- CCAGCAGCCGCGGTAATACG-3′	[24]
16S RNA Reverse	5′- ATCGGCTACCTTGTTACGACTTC-3′	

Measurement of Humidity and Temperature

Indoor air temperature and relative air humidity were monitored using Q-Trak IAQ Monitor (TSI Model 8551) and VelociCalc (TSI Incorporated, 500 Cardigan Road, Shoreview, MN 55126, USA) at the study sites [26].

Statistical Analysis

SPSS software version 16 was used for analysing the data obtained. The normality of the data was assessed using Kolmogorov–Smirnov and Shapiro–Wilk test. One-way ANOVA test, Kruskal–Wallis test and Mann–Whitney U test were used to determine the differences and similarities between schools and sampling sites. The significant differences of airborne concentrations of bacteria and fungi between the indoor air with occupants and without occupants were analysed by means of the sign-ranked test. Spearman's correlation analyses were performed to determine the relationship between relative humidity and temperature with the bioaerosol concentrations in the schools. P values of 0.05 or less were considered to be significant.

Results

Table 2 shows a summary of temperature and relative humidity measured in the schools. The average indoor temperatures and relative humidity were 25.8–34.0°C and 74.4–77.2%, respectively, while the average outdoor temperatures ranged from 26.1°C to 34.3°C and relative humidity ranged from 74.2% to 77.4%. The mean temperature indoors was lower than the corresponding outdoor level (Z –3.243, $p < 0.01$), while there was no significant difference between the mean relative humidity indoors and outdoors (Z –0.046, $p > 0.05$). The temperature and relative humidity showed inverse correlation (r –0.802, $p < 0.05$) so that high temperature was correlated with low humidity. There was no significant difference in the temperature (χ^2 3.246, $p > 0.05$) between the schools but the relative humidity between indoor classrooms showed significant difference (χ^2 21.21, $p < 0.01$). There was a moderate inverse correlation between relative humidity and bacterial concentrations (r −0.351, $p < 0.05$), while no significant correlation was found between relative humidity and fungal concentrations (r −0.06, $p > 0.05$).

This study has shown that bacteria and fungi were present in the indoor air of the classrooms. Prior to identification, bacterial and fungal colonies isolated on 150 Petri plates were counted as CFU and adjusted for the statistical possibility using the positive hole correction table. The bacterial and fungal bioaerosol concentrations were then quantified as CFU/m^3 to determine if the maximum bioaerosol levels were exceeded. The bacteria concentrations indoors without occupants were significantly lower than the indoors with occupants (Z −3.456, $p < 0.05$) and the outdoor air (Z −2.734, $p < 0.05$); while no significant difference exist between the bacteria

Table 2. Temperature (°C) and relative humidity (%) in the indoor air between November 2008 and June 2009

School number	Sampling location	N	Temperature	Relative humidity
			Mean (95% CI) Median Min Max	Mean (95% CI) Median Min Max
Primary School 1	Classrooms	9	29.4 (27.3–31.6) 28.2 26.9 33.5	75.5 (74.5–76.5) 75.7 73.8 76.9
	Corridor	4	32.0 (31.7–32.4) 32.1 31.5 32.3	75.0 (74.3–75.8) 74.8 74.4 76.1
	Canteen	2	27.0 (26.6–27.3) 27.0 26.7 27.2	75.7 (75.3–76.0) 75.7 75.4 75.9
Primary School 2	Classrooms	9	29.9 (27.3–32.4) 28.9 26.8 34.0	75.6 (74.2–77.1) 76.3 73.0 77.1
	Corridor	4	33.5 (32.7–34.3) 33.6 32.3 34.3	76.2 (75.5–76.9) 76.1 75.5 77.1
	Canteen	2	26.1 (25.8–26.5) 26.1 26.1 26.2	75.3 (74.7–75.9) 75.3 74.9 75.7
Primary School 3	Classrooms	9	28.4 (26.4–30.4) 28.3 25.8 31.6	75.2 (73.6–76.8) 75.6 72.0 77.4
	Corridor	4	32.3 (31.8–32.8) 32.1 31.8 33.2	73.9 (72.1–75.6) 74.5 71.4 75.1
	Canteen	2	26.6 (26.0–27.2) 26.6 26.2 27.1	74.1 (74.0–74.1) 74.1 74.0 74.1
Primary School 4	Classrooms	9	28.4 (26.6–30.2) 28.9 26.1 31.2	75.8 (74.6–76.9) 76.0 74.0 77.0
	Corridor	4	33.9 (33.6–34.2) 33.8 33.6 34.3	77.0 (76.6–77.3) 77.0 76.6 77.4
	Canteen	2	27.2 (27.1–27.3) 27.2 27.1 27.3	74.2 (74.0–74.4) 74.2 74.1 74.3
Primary School 5	Classrooms	9	29.0 (28.0–30.0) 28.9 28.0 30.8	77.2 (76.6–77.8) 77.0 76.5 78.3
	Corridor	4	28.1 (27.5–28.7) 28.3 27.2 28.6	77.5 (76.9–78.2) 77.5 77.0 78.6
	Canteen	2	29.1 (28.8–29.4) 29.1 28.9 29.3	77.7 (77.6–77.7) 77.7 77.6 77.7
Average	Indoor		29.0 (27.0–31.0) 28.7 25.8 34.0	75.8 (74.4–77.2) 76.3 72.0 78.3
	Outdoor		31.0 (28.1–33.9) 32.1 26.1 34.3	75.8 (74.2–77.4) 75.7 71.4 78.6

N: number of samples.

Table 3. Bacteria concentration at sampling stations in selected primary schools between November 2008 and June 2009

School number	Sampling location	*N*	Bacteria concentration with occupant (CFU/m^3)				Bacteria concentration without occupant (CFU/m^3)			
			Mean (SD)	Median	Min	Max	Mean (SD)	Median	Min	Max
Primary School 1	Classrooms	9	2947 (752)	2952	2090	3795	1293 (802)	1558	0215	2240
	Corridor[a]	4	–	–	–	–	3665 (324)	3700	3325	3970
	Canteen[b]	2	2050	–	–	–	0555	–	–	–
	I/O ratio		0.8				0.35			
Primary School 2	Classrooms	9	0775 (642)	0558	0290	1695	0343 (161)	0298	0170	0555
	Corridor[a]	4	–	–	–	–	1183 (929)	0815	0495	2240
	Canteen[b]	2	750	–	–	–	420	–	–	–
	I/O ratio		0.66				0.29			
Primary School 3	Classrooms	9	1700 (938)	1475	0820	3030	0635 (325)	0650	0285	1195
	Corridor[a]	4	–	–	–	–	1383 (774)	1265	0675	2210
	Canteen[b]	2	485	–	–	–	350	–	–	–
	I/O ratio		**1.23**				0.46			
Primary School 4	Classrooms	9	0512 (214)	0528	0260	0735	0246 (167)	0228	0100	0560
	Corridor[a]	4	–	–	–	–	0750 (646)	0415	0340	1495
	Canteen[b]	2	370	–	–	–	160	–	–	–
	I/O ratio		0.68				0.33			
Primary School 5	Classrooms	9	1351 (721)	1410	0420	2165	0443 (167)	0438	0245	0685
	Corridor[a]	4	–	–	–	–	0658 (572)	0458	0220	1495
	Canteen[b]	2	1035	–	–	–	400	–	–	–
	I/O ratio		**2.0**				0.67			
Average	Classrooms		1457 (1072)	1385	0260	3795	0592 (536)	0405	0100	2240
	Corridor[a]						1473 (1261)	1040	0220	3970
	Canteen[b]									
	I/O ratio		0.98				0.4			

[a]Bioaerosol sampling was conducted with no occupant.
[b]Sampling was done only one time, with occupant and without occupant.
N: number of samples.
I/O > 1 = Bold Values.

concentrations indoors with occupants present and outdoors (Z –0.159, $p > 0.05$). There was also no significant difference between the indoor air fungal concentrations with and without occupants (Z –1.830, $p > 0.05$) or between indoor air with occupants and outdoor air (Z –0.537, $p > 0.05$). Fungal concentrations in outdoor air were found to be significantly higher than in indoor air without occupants (Z –2.853, $p < 0.05$).

School 1 showed the highest bacteria concentrations both in the outdoor and indoor air while Schools 4 and 5 had the lowest bacterial concentrations in the indoor and outdoor air (Tables 3 and 4). As shown in Figure 1, the average indoor bacteria concentrations with occupants in all the schools were higher than the recommended level for bacteria [19], while the indoor bacteria concentrations with no occupants in all schools were within the recommended level except in Schools 1 and 3. At least one study location in all the schools showed bacteria concentrations higher than the recommended level except for School 4. Indoor bacteria concentrations with occupants ranged from 260 to 3795 CFU/m^3 with a mean of 1457 (SD1072) CFU/m^3; without occupants, the concentrations ranged from 100 to 2240 CFU/m^3. The concentrations in the outdoor air ranged from 220 to 3970 CFU/m^3.

The lowest indoor and outdoor fungal concentrations were found in School 5, while the highest indoor fungal concentration with occupants was found in School 3. School 1 showed the highest indoor fungal concentrations. Figure 2 showed the outdoor and indoor fungal concentrations with occupants in all the schools and these were above the recommended level except in School 5. Outdoor and indoor fungal bioaerosol concentrations with and without occupants ranged from 65 to 700 CFU/m^3 and 65 to 530 CFU/m^3, respectively, with mean of 351 (SD 196) CFU/m^3 for indoors with occupants and 233 (SD 138) CFU/m^3 for indoors without occupant. Outdoor fungal concentrations ranged from 140 to 1055 CFU/m^3 with an average of 401 (SD 235) CFU/m^3.

Further investigation was done on the indoor-to-outdoor (I/O) ratios of airborne bacteria and fungi in order to determine if indoor spaces were contaminated with airborne microorganisms [21,27]. Ratios of I/O

Table 4. Fungi concentration at sampling stations in selected primary schools between November 2008 and June 2009

School number	Sampling location	*N*	Fungi concentration with occupants (CFU/m^3)				Fungi concentration without occupants (CFU/m^3)			
			Mean (SD)	Median	Min	Max	Mean (SD)	Median	Min	Max
Primary School 1	Classrooms	9	446 (234)	495	135	700	403 (124)	430	185	520
	Corridor[a]	4	–	–	–	–	520 (434)	443	140	1055
	Canteen[b]	2	188	–	–	–	120	–	–	–
	I/O ratio		0.86				0.78			
Primary School 2	Classrooms	9	385 (137)	355	235	540	200 (099)	183	080	325
	Corridor[a]	4	–	–	–	–	403 (208)	402	195	610
	Canteen[b]	2	415	–	–	–	300	–	–	–
	I/O ratio		0.96				0.5			
Primary School 3	Classrooms	9	501 (137)	485	365	655	210 (066)	220	115	295
	Corridor[a]	4	–	–	–		484 (171)	465	305	700
	Canteen[b]	2	355	–	–		250	–	–	–
	I/O ratio		**1.04**				0.43			
Primary School 4	Classrooms	9	293 (142)	305	080	465	208 (166)	163	065	530
	Corridor[a]	4	–	–	–	–	335 (140)	333	185	490
	Canteen[b]	2	125	–	–	–	070	–	–	–
	I/O ratio		0.87				0.62			
Primary School 5	Classrooms	9	121 (061)	115	065	700	142 (079)	133	065	245
	Corridor[a]	4	–	–	–		266 (117)	230	170	435
	Canteen[b]	2	305	–	–		070	–	–	–
	I/O ratio		0.45				0.53			
Average	Classrooms		351 (196)	351	065	700	233 (138)	210	65	530
	Corridor[a]						401 (235)	353	140	1055
	Canteen[b]									
	I/O ratio		0.86				0.58			

[a]Bioaerosol sampling was conducted with no occupant.
[b]Sampling was done only one time, with occupant and without occupant.
N: number of samples.
I/O > 1 = Bold Values.

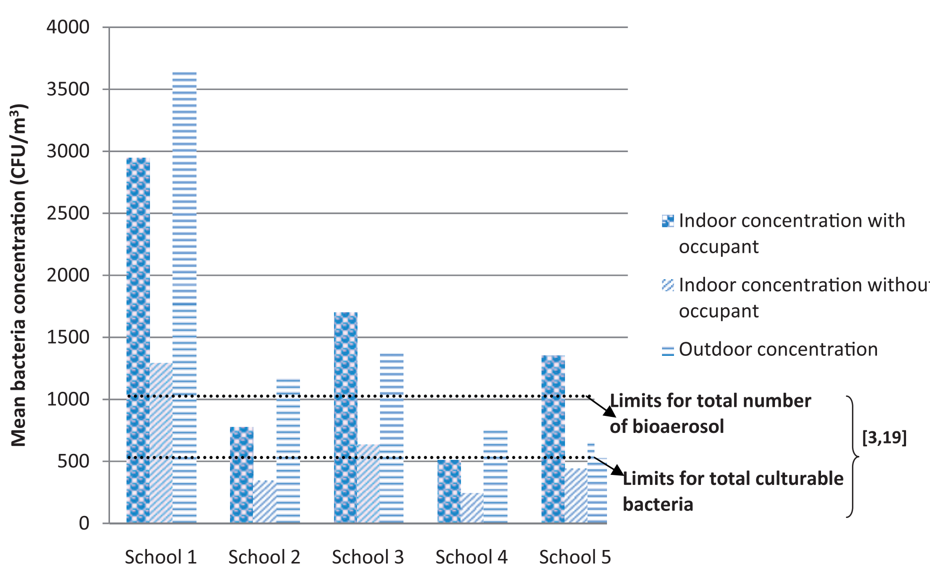

Fig. 1. Mean indoor and outdoor bacteria concentrations with and without indoor occupants.

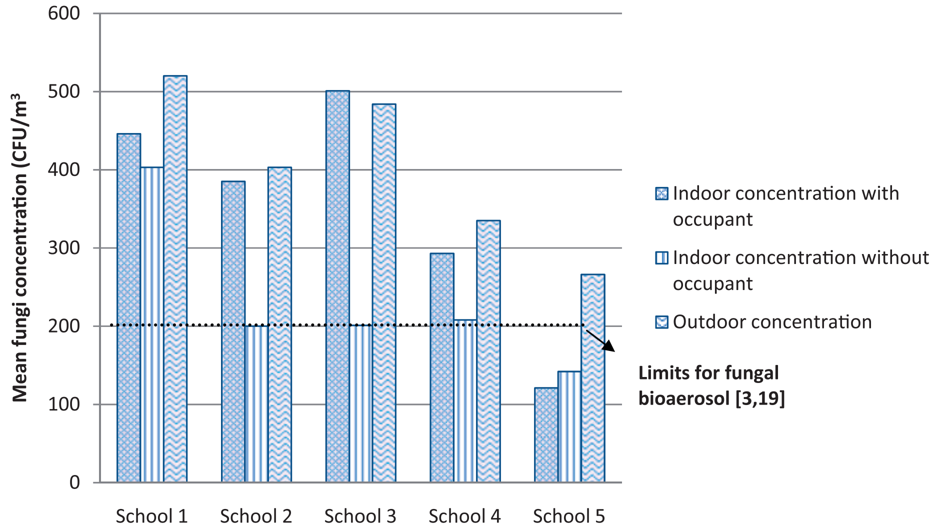

Fig. 2. Mean indoor and outdoor fungi concentrations with and without indoor occupants.

for both bacteria and fungi concentrations in all the sampling sites were below 1.0, except in School 5 and School 3 (Tables 3 and 4). The lowest I/O ratios for bacteria and fungi were found in School 2 and School 5, respectively.

Identification of Bacterial and Fungal Bioaerosols

Frequently isolated bacterial and fungal colonies were further identified using both conventional and molecular techniques of laboratory diagnosis. Table 5 shows the colony numbers and percentage of identified bacteria isolated in the study. Throughout the study period, 33 bacterial species and 15 fungal genera were identified. More than half (54.5%) of the most frequently isolated bacteria were Gram-positive cocci (*Staphyloccoccus* spp. and *Micrococcus* spp.) followed by 19.4% Gram-negative bacilli or coccobacilli (*Pseudomonas* spp., *Enterobacteriacea* and *Acinetobacter* spp.), 18.2% Gram-positive rod (*Bacillus* spp.) and 3.03% non-spore forming Gram-positive rods (*Corynebacterium* spp.). Overall, bacteria species isolated in this study have also been found in other studies, except for *Terribacillus* spp., which was found for the first time in schools in this study.

The most common indoor airborne fungal genera isolated were *Aspergillus*, *Penicillium*, *Fusarium*, *Rhizopus* and *Zygomycetes* (Table 6). Results showed that in all the investigated indoor air samples the predominant genus was *Aspergillus* while in the outdoor air genus *Penicillum* was commonly found. Other identified genera included *Alternaria*, *Cladosporium* and *Gliocladium*. None of these fungal genera had concentrations of more than 50 CFU/m^3.

Discussion

In this study the average indoor temperature and relative humidity were higher in comparison with those in cold countries due to less variation in the temperature and climate that prevail throughout the year in Malaysia. Studies in cold countries found that the outdoor temperatures and relative humidity were lower than corresponding indoor levels [16], while in this study, results showed that the indoor temperatures were lower than the corresponding outdoor levels and no significant difference was observed in the relative humidity between indoor and outdoor environments. Our results also showed that indoor temperature had no effect on the generative pattern of airborne bacteria and fungi, but indoor relative humidity did have an effect on bacterial concentration. However, some studies have been controversial on the effect of temperature and relative humidity on indoor airborne microorganisms [28]. Due to differences between climates, findings on IAQ and bio-aerosols reported in cold climates may not be applicable to Malaysia.

Furthermore, this study investigated the types and concentrations of viable bacterial and fungal bioaerosols in primary schools in Malaysia. It was shown that indoor air concentrations of bacteria in all the schools except for School 4 exceeded the recommended level established by the National Institute of Occupational Safety and Health (NIOSH) [29] and the American Conference of Governmental Industrial Hygienists (ACGIH) [19] at 1000 CFU/m^3 of total number of bioaerosol particles in which the culture count for total bacteria should not exceed 500 CFU/m^3 [5,19]. The ACGIH and Rao et al.

Table 5. Colony numbers and percentage of identified bacteria isolated

Genera and species		N	Percentage (%)
Acinetobacter		4	2.42
A. calcoaceticus	Gram-negative	5	3.03
A. schindleri	Gram-positive		
Bacillus			
B. firmus	Gram-positive	3	1.82
B. flexus	Gram-positive	2	1.21
B. luciferensis	Gram-positive	1	0.61
B. megaterium	Gram-negative	2	1.21
B. pumilus	Gram-negative	9	5.45
B. shandongensis	Gram-negative	3	1.82
B. subtilis	Gram-negative	10	6.06
Corynebacterium			
C. aurimucosum	Gram-positive	2	1.21
C. urealyticum	Gram-positive	3	1.82
Enterobacteriaceae			
Pantoae agglomerans	Gram-positive	3	1.82
Pantoae dispersa	Gram-positive	2	1.21
Enterobacter aerogenes	Gram-positive	4	2.42
Micrococcus			
M. luteus	Gram-positive	2	1.21
Moraxella spp.			
Pseudomonas			
P. putida	Gram-negative	2	1.21
P. aeruginosa		2	1.21
P. stutzeri		3	1.82
Staphylococcus		9	5.45
S. aureus		21	12.73
S. chromogenes		3	1.82
S. cohnii subsp urealyticum		4	2.42
S. cohnii susp. cohnii		5	3.03
S. epidermidis		9	5.45
S. haemolyticus		7	4.24
S. hominis		6	3.64
S. hyicus		5	3.03
S. intermedius		3	1.82
S. saprophyticus		5	3.03
S. warneri		7	4.24
Uncultured *staphylococcus*		13	7.88
Stenotrophomonas maltophilia		2	1.21
Terribacillus		4	2.42

[19,20] have recommended 200 CFU/m^3 as a guideline for indoor fungal bioaerosols: our results showed that none of the schools except for School 5 was within this recommended level.

Schools 1 and 3 had the highest indoor bacterial and fungal concentrations, respectively. Both the bacterial and fungal concentrations in these schools exceeded the recommended limit and thus should be considered unacceptable for normal occupation due to poor hygienic air quality [15,30]. Based on the walk-through building inspection conducted, the unhygienic conditions of School 1 and School 3 were obvious compared with the other schools. These two schools had dusty floors and mouldy indoor surfaces such as furniture and ceilings. Food remnants and rubbish wastes were not disposed of regularly. These conditions could be improved by frequent cleaning including wiping of surfaces, floor cleaning and mopping with a cleaning agent to reduce bacteria and fungi concentrations [31].

Investigation of air samples from schools in this study showed that the average indoor bacteria concentrations with occupants were significantly higher than without occupants, suggesting that the presence of humans could be a source of the bioaerosols in the indoor environments. Activities such as talking, sneezing and coughing could generate and increase the human transmission of indoor

Table 6. Number and percentage of identified fungi isolated

Genera	N	Percentage (%)
Actinomyces	3	3.95
Alternaria spp.	5	6.58
Aspergillus		
A. Flavus	8	10.53
A. nidulans	6	7.89
Cephalosporium spp.	4	5.26
Cladosporium spp.	5	6.58
Curvularia spp.	3	3.95
Fusarium spp.	6	7.89
Gliocladium spp.	5	6.58
Helminthosporium spp.	4	5.26
Penicillium spp.	7	9.21
Rhizopus spp.	6	7.89
Syncephalastrum spp.	3	3.95
Ulocladium spp.	3	3.95
Verticillium spp.	2	2.63
Zygomycetes	6	7.89

bioaerosols [1,5]. However, no significant difference was found in the indoor fungal concentrations with and without occupants. Fungal concentration in the outdoor air was found to be significantly higher than indoor air without occupants, as explained by the presence of sources from outdoor plants, soil and vegetation.

For all schools investigated, the indoor airborne bacteria and fungi concentrations without occupants were demonstrated to be lower than the outdoor concentrations. This showed that the building (or activities within the building) had contributed to the increased bioaerosol concentrations indoors. On the other hand, I/O ratio for airborne bacteria concentrations with occupants in School 5 exceeded 1.0. The I/O ratio for both bacteria and fungi concentrations in School 3 exceeded 1.0. In this case, the indoor air could be contaminated with airborne microorganisms from outdoor airborne microorganisms by natural ventilation or due to inside generative sources [11,21,32]. On-site observations in School 3 showed the presence of dampness on the indoor surfaces where mould can easily proliferate and thus contribute to the increase in the indoor microbial concentrations [16]. Since *Aspergillus* spp. was dominant in this indoor air, therefore, it was consistent with the previous findings that this fungal species would mainly grow indoors [33,34].

Further investigations on bacterial bioaerosols in indoor air showed that most of the bacteria species isolated by this study were also found in other studies except *Terribacillu*s spp., which has been found in this study alone. Gram-positive cocci were the dominant bacterial species isolated during the study period. This finding was consistent with other studies conducted in schools, offices and residential buildings in Europe, Poland, Korea and Turkey as staphylococci are microorganisms that are widespread in nature [6,11,21,35]. However, detection of these microorganisms at a high level would indicate high density occupancy and inadequate ventilation [29].

Bacillus spp. accounted for the next most commonly isolated bacteria in this study. Members of the Bacillus genus are mostly aerobic saprophytes and endospore formers and are widely distributed in the natural environment, but some species are opportunistic or obligate pathogens of animals, including humans and other mammals [6,36]. *B. subtilis* was the most prominent *Bacillus* spp. isolated from the canteen samples, indicating a potential source of food contamination, but this species rarely causes food poisoning [37]. *Corynebacterium* spp. are Gram-positive non-spore-forming rods that are prominently isolated as many species of the *Corynebacteria* exist as a part of the normal flora of the skin and mucous membranes in human and mammals [25].

The third most common isolated was Gram-negative bacteria. Gram-negative bacteria are believed to have more harmful effect due to endotoxin production, which can cause inflammation of the airways and elicit asthmatic attacks and bronchial hyper-reactivity [4]. Endotoxins are normally shed by bacteria in household dust. Gram-negative bacilli or coccobacilli accounted for 21.8% of bacteria samples isolated by this study. *Stenotrophomas maltophilia* and *Moraxella* spp. isolated in this study were also found in indoor air in central and eastern Europe [35]. Further studies in preventing and minimizing exposure to these bacteria are recommended in the future. Despite this, some researchers believe that microbial exposures, and particularly exposure to endotoxin early in life, may protect children from developing atopy and allergic asthma; however, the mechanisms are not well understood [38].

For airborne fungi, the most frequently isolated genera during the study period were Aspergillus, Penicillium, Fusarium and Rhizopus. The other identified genera included Alternaria, Cladosporium and Gliocladium. Alternaria and Cladosporium are considered to be the most important indoor airborne fungal allergens while Penicilium and Aspergillus are significant indoor allergens [15,39]. The identification of genus Aspergillus up to species level should be the focus of future studies, since some species of Aspergillus such as *A. flavus*, *A. fumigatus* and *A. versicolor* have allergenic, toxigenic and infectious effects [15,40]. According to the recommendations of Rao et al. [20], air samples with fungi concentrations of $\leq$150 CFU/m^3 would be acceptable if they contain several fungal species. Considering this level of acceptance, indoor air was found to be satisfactory only in School 5. Fungal concentrations in the indoor air of Schools 1, 2 and 4 were not acceptable since the fungal concentrations were $>$150 CFU/m^3 and the predominant genus was Aspergillus. Most of these fungal genera were also found in other studies conducted in Turkey, Poland and Australia in which Aspergillus and Penicillium had been recognized as a significant indoor allergens [6,11,21,35].

Limitations of the Study

Since the design of this study is a cross-sectional, the bioaerosol collection methods were 'grab sampling' techniques and represent only approximate transient microbial types and concentrations in the specified atmosphere at a given point of time, and therefore would limit the scope of generalization for a tropical characterization due to the hot and humid weather conditions. The profile of the CFU versus sampled air volume for a few sampling locations suggests the need to sample a range of volumes, as CFU counts approaching 200 could produce a significant masking effect. The microbiological air sampler manual provided by the International Pbi S.p.a., (2003) recommended 200 litres as optimum standard volume for measurements in homes, schools and office buildings. In addition, the lack of reference limit values for bioaerosols in tropical countries has hindered the interpretation of results obtained. Until now, Malaysia has no established legal standards related to airborne microorganisms [41].

Conclusion

High indoor bioaerosol concentrations were found in Schools 1 and 3, reflecting their state of cleanliness and over-crowding. Outdoor air bioaerosol concentrations were strongly affected by the nearby regional vegetation activities. The indoor levels of bacteria and fungi concentrations in schools with occupants were significantly higher than those without occupants due to contamination by the occupants. The ratio of indoor to outdoor bioaerosol concentrations was below 1.0 except for bacteria concentrations in School 5 and for fungi concentrations in School 3, indicating the state of indoor conditions that would provide favourable conditions for both bacteria and fungal growth. More than half of bacteria samples (56%) had a concentration exceeding the ACGIH and World Health Organization (WHO) recommended level of 500 CFU/m^3, and only 33.8% of fungal samples were within recommended level of 200 CFU/m^3. This indicated poor microbial indoor air quality in the schools that could have a harmful effect on children's health.

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