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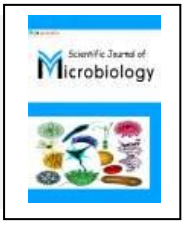

Original article

Characterization, identification and comparison of indoor microbial fungi and bacteria between urban and suburban schools in Penang, Malaysia

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ABSTRACT

School environment plays a big role in influencing a child's health and well being. This is due to the known fact that children commonly spend a long duration of time in school. Children's growing lungs present a large surface area that enables pollutants to be easily absorbed. Therefore, environmental condition in school is one of the main. Since studies on indoor microbial fungi and bacteria is limited, this preliminary study was aimed to determine the concentration of fungi and bacteria in the indoor air in selected locations in the schools and to compare them between the urban and suburban schools. Duo SAS Super 360 Microbiological Air Sampler was used to collect air samples from 8 urban and 2 suburban schools in Penang. Temperature, relative humidity and carbon dioxide level were measured using TSI Q-TRAK Plus IAQ Monitor Model 8554. Fungi and bacteria were identified using Lactophenol Blue staining for fungi and Polymerase Chain Reaction (PCR) for bacteria. Eight locations in each school were selected as sampling points. All eight urban schools exceeded the permitted limit of microbial fungi concentration recommended by ACGIH. Aspergillus has the highest occurrence range (75-100%) followed by Penicillium (37-75%) and Rhizopus (0-75%). Highest occurrence range of microbial bacteria was Staphylococcus Sp. (62-100%), Bacillus sp. (50-100%) and Corynebacterium sp. (37-88%). Microbial fungi

concentrations were highest in sports equipment room in 6 urban schools and lowest in canteen in all 8 urban schools. However, microbial bacteria concentration was high in both classrooms and sports room but lowest in the canteen. There was significant difference between fungal concentration in urban and suburban schools ($p < 0.05$). A significant positive correlation was observed between fungal concentration and carbon dioxide ($p < 0.05$) in classrooms. In conclusion, carbon dioxide influences the concentration of fungi in the classroom. This could be caused by occupancy in the classroom.

1. Introduction

Issues on the indoor air quality have always been highlighted in the past years. Many aspects related to air quality such as air contaminants, health effects and surrounding factors have been studied and was concluded to be given more attention. Most common air contaminants which are within the health risk category are such as radon, gases from landfill or waste sites, formaldehyde, volatile organic compounds (VOC), carbon monoxide (CO), nitrogen oxide (NO2), metabolic gases and airborne microorganisms (WHO., 2000). Exposure to such air contaminants provides health risks such as allergy, airway infections, asthma, rhinitis, sick building syndrome (SBS) and many others (Ross et al.,2000; Kalogerakis et al., 2005).

There are many factors contributing to the concentration and types of airborne microorganisms found in indoor air. The most common environmental factors are meteorological parameters such as wind, relative humidity, temperature, changing climate, altitude and vegetation (Asan et al., 2002). Human activity and land use such as land fill, quarry, industrial and mining areas also contributes to the airborne microorganism found in indoor air. Studies has reported that condition in buildings such as moisture content of building materials (Pasanen et al., 2000), relative humidity in a building, outdoor microbial concentration, the rate of air exchange, number of people and pets in the building and type of human activities (Pasanen et al., 1997) that take place influences the concentration of airborne microorganism in indoor environment. According to a study by Rajasekar et al (2011), indoor airborne bacteria concentration was influenced by occupancy level while indoor fungi concentration was influenced by relative humidity.

Lately, airborne microorganism was reported to have caused many health effects (Kim et al., 2011; Hussin et al.,2011) which answers the need to do more research on these sources of air contaminant. Airborne microorganism such as viruses, bacteria, fungi and their products is particular indoor biological air contaminant that causes adverse health effect to occupants. Airborne fungal exposure in particular causes variety of health outcome such as development of sensitivities, allergic, toxigenic, asthma and respiratory symptoms (Taskinen et al., 1999). Commonly, the most vulnerable group to airborne fungi are children and immunosuppressant occupants. Therefore, airborne fungal exposures in schools are given particular attention considering the age and susceptibly of the occupants.

Airborne fungal causes growth of filamentous fungi and spores when facilitated with sufficient moisture in indoor environment. Fungal spores fluctuate in concentration seasonally depending on moisture content, temperature and many other environmental factors (Bartlet., 2004). According to Haleem et al (2009), Ascomycotina, Basidiomycotina and anamorphic fungi cause allergy related problems.

Another main biological pollutant that is given attention in indoor air quality study is bacteria. The main sources of bacteria in the indoor environment are outdoor air, people and indoor bacterial growth. Bacteria from outdoor air and those resourcing from people are considered to be harmless or also termed as normal flora. However, some bacteria growing actively or accumulating in the indoor environment may affect health (Samson et al., 1994).

The main objective of this study was to determine the concentration of fungi and bacteria in the indoor air in selected locations in the schools and to compare them between the urban and suburban schools.

2. Materials and methods

2.1. Study design and location

This cross-sectional study was conducted between October 2010 and March 2011 in Penang, Malaysia. Penang is divided into mainland and island. Out of 125 schools in Penang, ten secondary schools were randomly selected after excluding single gender schools, religious schools and technical schools. From the ten selected schools, there were eight urban and two suburban schools. Five schools were selected from mainland and another five were from island (Fig. 1).

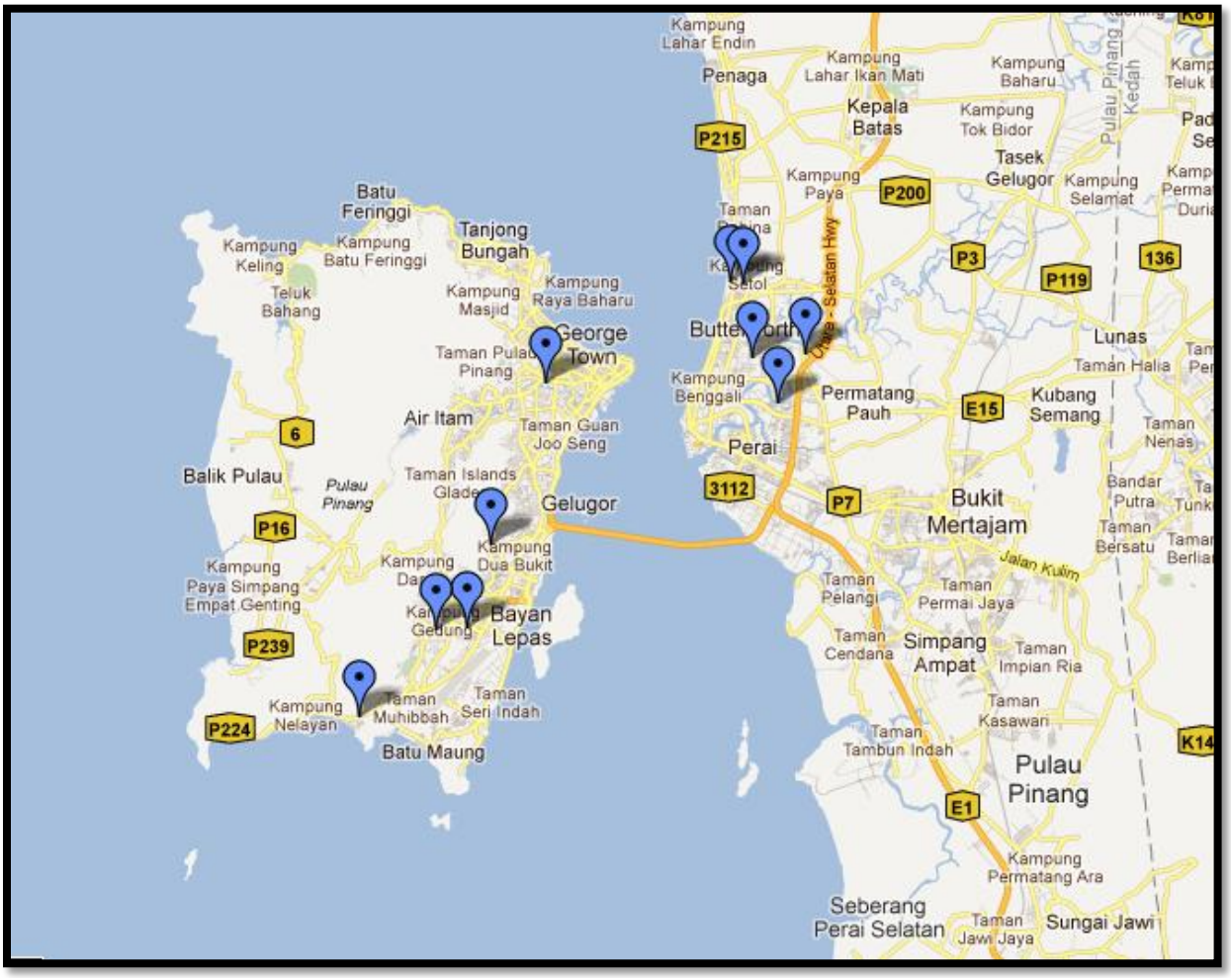

Fig. 1. Location of selected schools in mainland and island.

2.2. Environmental parameter

A walkthrough inspection took place surrounding the school buildings to determine the sampling points. Observation was based on the land use and human activity, age of building and the type of ventilation used. Land use and human activity between 400m radius is studied for each school. Measurement was taken for indoor air temperature, carbon dioxide and relative air humidity during school hours using Q-Trak IAQ Monitor (TSI Model 8551) for an hour.

2.3. Sampling locations

Eight sampling locations were identified namely 4 classroom, canteen, library, science laboratory and sports equipment room as children spend at least few hours in these places in a week. Three classrooms and two science laboratory were selected randomly as all the selected schools consists of more than seven form two classes and

these children use at least two science laboratories for their science subject. Duo SAS Super 360 microbiological air sampler (International P.B.I. S.p.A., Via Novara, 8920153 Milano, Italia) was used for sampling of airborne fungi and bacteria. This device has two heads with 400 holes on each head to ensure air particle suction. The sampled air was embedded on petri dishes that were placed in the contact plate holder with suitable agar media. The media used for airborne fungal sampling was Sabouroud Dextrose Agar (SDA) and Tryptic Soy Agar (TSA) for airborne bacteria (Kim and Kim., 2007). The air sampling duration for each location was 2 minutes for 200 liters of air as stated in the manual of Duo SAS Super 360 microbiological air sampler (International Pbi S.p.a., 2003) Air sampling took place in the middle of the sampling locations at 1 meter height. Samples were collected during the weekdays between 0900 and 1700 hours according to the Form two class sessions. Sampling was carried out in duplicate. Prior to air sampling, the inside part of the air sampler was sterilized with 70% ethanol swab to ensure there are no other contaminants.

2.4. Microbiological analysis

The sampled petri plates were incubated aerobically in incubator for 3-5 days at 20-25°C (Husna et al., 2011). Within the third and the fifth day of incubation, colonies of bacteria and fungi can be seen clearly under naked eyes. Prior to Colony Forming Unit (CFU) calculation, the number of colonies counted on the agar surface was corrected for statistical possibilities of multiple particles passing through the same hole according to the Positive Hole Correction Table given with the manual. Concentration or CFU/m^3 was calculated using formula as below:

$$X = \frac{Pr \times 1000}{V}$$

Where X is CFU per 1000 litres of air (1 cubic metre) and Pr is probable count obtained by positive hole correction. V is volume of sampled air (200 litres of air).

Identification of fungal bio-aerosol was performed till genus level using basic morphological and microscopic observation. Colonies that are different morphologically are cultured on a new SDA media plate and incubated for 3-5 days before thorough observation based on colour, growth characterization and surface appearance. Further observation was performed using cellophane tape mount and lactophenol cotton blue as stain. Slides were prepared as viewed under the microscope.

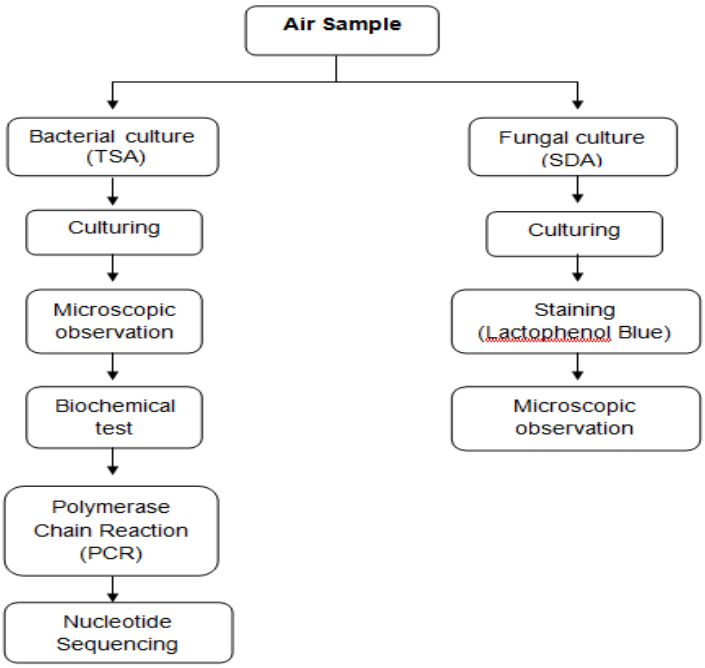

Fig. 2. Flow chart for microbiological analysis

Identification bacteria bio-aerosol involved culturing and preservation of bacterial stock, preliminary microscopic identification, basic biochemical testing (Bergey's Manual of Determinative Bacteriology), Polymerase

Chain Reaction (PCR) using 16S RNA gene which was the universal bacterial identification method (Husna et al., 2011) and sequencing (Fig. 2).

2.5. Statistical analysis

Data obtained were analyzed using Statistical Package for Social Sciences (SPSS) version 19. Analysis was done using univariate, bivariate and multivariate testing on variables involved in this study.

3. Results

Out of the ten selected schools, the oldest school and building sampled was School 4 which was built in 1887 and the latest was classrooms in School 5 (Table 1). All the selected schools were surrounded by residential area. However, School 2 was surrounded by industrial area and a graveyard was within 200m from School 4. A landfill was within 100m from School 9 (Table 1). School 1 to 8 were urban schools while School 9 and 10 were suburban schools.

Average airborne fungal CFU in urban school ranged between 43-94 cfu/m3 and the average of fungal CFU in all 8 urban schools were 464 cfu/m3. Airborne fungal CFU was the highest in School 2 (20.3%) while the lowest count was in School 6 (9%). On the other hand, suburban schools consist of 2 schools whereby average fungal count in both schools was 56 cfu/m3. Total fungal CFU in School 9 was 32 cfu/m3 (57.1%) while in School 10; it was 24 cfu/m3 (42.8%) which is relatively lower than School 9 (Table 2).

The average sum of CFU for all 8 urban schools was 387. The highest bacterial CFU was in School 8 (66 cfu/m3) whereby it contributes 17.1% to the total average sum of CFU for all the 8 urban schools. The lowest count was in School 3 (41 cfu/m3) and it contributes 10.5% to the total average. The other schools were within the range of 42 cfu/m3 and 51 cfu/m3 and the percentage ranges from 10.9% and 13.1%. Suburban schools consist of 2 schools and the average bacterial CFU in suburban school was 85 cfu/m3. School 10 was higher (44 cfu/m3) whereby it contributes 51.8% to the total sum of CFU compared to School 9 (41 cfu/m3) in which the percentage was 48.2% (Table 3).

Air sampling took place at 8 indoor area (3 classrooms, 2 science laboratories, library, canteen and sports equipment room) belonging to each school. Figure 1 showed the summary of bioaerosol cfu in all locations within the selected schools. The mean was calculated for classrooms and science laboratories. The highest count of airborne fungi CFU was in the sports equipment room in School 2, 3, 5, 6, 7 and 8. In School 1, the highest count was in classroom while in School 4 and 9, it was in library. In School 10, the highest CFU count was in canteen. The lowest count of airborne fungi within 5 locations in all 8 urban schools was the canteen. The lowest count for both School 9 and 10 was in the classroom.

At least 1 location was above the recommended level of fungi bioaerosol cfu in all 8 urban schools. However, none of the locations in suburban school was above the recommended level. All 5 locations in School 2 were above 250 cfu which was the recommended level (ACGIH, 1989). 4 locations in School 8, 3 locations in School 4 and 2 locations in School 3, 5, 6 and 7 were above recommended level.

Sports equipment room in School 2, 3, 4, 5, 6 and 7 showed the highest count of bacterial CFU. Classroom showed the highest CFU count in School 1 and 8. In School 9, highest bacterial count was in the canteen while in School 10, there were 2 locations; classroom and the library. Meanwhile, in School 1, 2, 4, 6 and 8, the lowest bacterial CFU count was in the school canteen. The lowest count of bacterial CFU in School 1, 5, 9 and 10 was in science laboratory. However the lowest count within all 8 locations in School 3 was in classroom (Figure 4).

Eleven different genera were identified and some could not be identified. Aspergillus sp. shows relatively high occurrence compared to other genera whereby it ranges within 75-100%. Aspergillus sp. was found in all eight locations in School 1 and 6 while in School 2, 5, 7 and 10, their occurrences were in 6 locations. The lowest occurring airborne fungal genus was Trichoderma sp. where it only occurred in 1 location (12.5%) in School 9. Fungal genera such as Aspergillus sp. and Penicillium sp. was found in abundance in all 10 schools. However, Verticillium sp., Paecillomyces sp. and Geotrichum sp. were only found in urban schools. On the other hand, Trichoderma sp., being the lowest occurring genus was found only in School 9 which was a suburban school (Table 4). Staphylococcus aureus was with relatively high occurrence ranging from 62.5-100% in all the 10 schools. The highest range (100%) was identified to be in School 9 while the lowest range (62.5%) was in School 5, 8 and 10. Staphylococcus sp. was found within a moderate range of 25-75% whereby the highest occurrence was in School 3, 7 and 9 (75%).

Table 1
Characteristics of Selected Schools

School	Surrounding land use and human activities	Building age
School 1	Residential area, industrial area, main highway, river	1992
School 2	Residential area, industrial area, food company, rubber company, flats	1966
School 3	Residential area, beach, main road, mosque	1986
School 4	Residential area, graveyard, petrol station	1887
School 5	Residential area, main road	1964, classrooms-2003
School 6	Residential area, forest	2001
School 7	Residential area, Chinese temple, commercial area, busy road	Classroom, library-1993, other-1967
School 8	Residential area, primary school, hospital, mosque, construction area	2003
School 9	Residential area, landfill, highway	1995
School 10	Residential area, primary schools, mosque, forest	Sports room-1989, Classrooms, library-1990, Science lab-2001

Table 2
Average concentration of fungi bioaerosol in urban and suburban schools.

Schools	Average Colony Forming Unit (CFU)	
	Urban	Suburban
School 1	43 (9.3)	
School 2	94 (20.3)	
School 3	43 (9.3)	
School 4	57 (12.3)	
School 5	55 (11.9)	
School 6	42 (9.0)	
School 7	45 (9.6)	
School 8	85 (18.3)	
School 9		32 (57.1)
School 10		24 (42.8)
Total	464 (100)	56 (100)

Table 3
Average concentration of bacteria bioaerosol in urban and suburban schools

Schools	Average Colony Forming Unit (CFU)	
	Urban	Suburban
School 1	47 (12.2)	
School 2	47 (12.2)	
School 3	41 (10.5)	
School 4	48 (12.4)	
School 5	51 (13.1)	
School 6	42 (10.9)	
School 7	45 (11.6)	
School 8	66 (17.1)	
School 9		41 (48.2)
School 10		44 (51.8)
Total	**387 (100)**	**85 (100)**

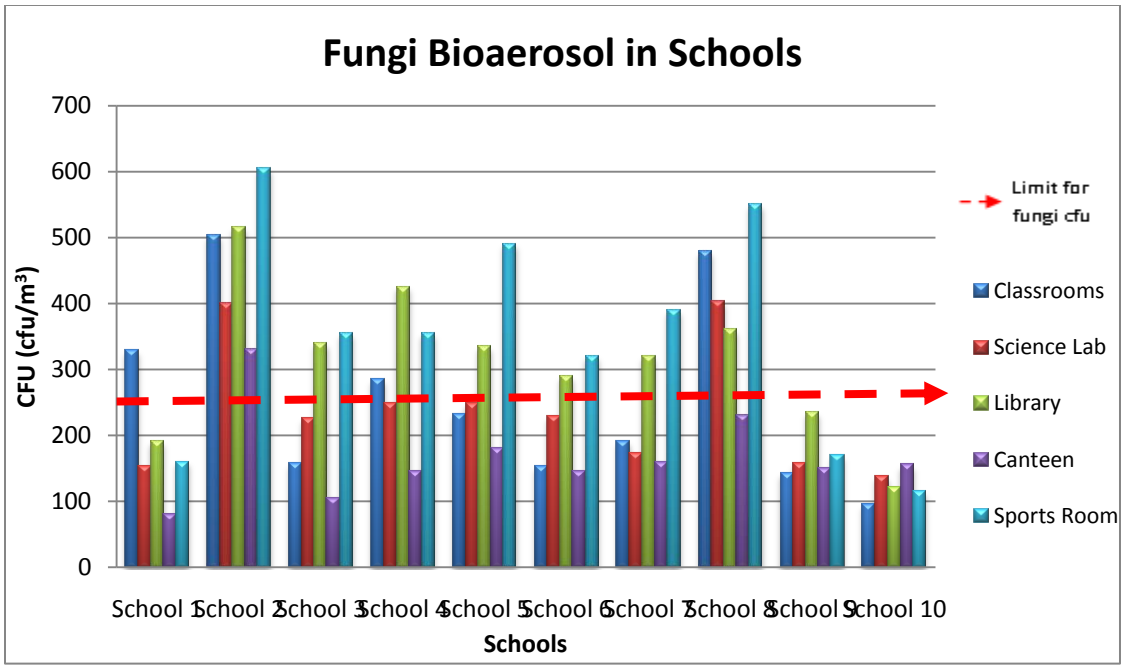

Fig. 3. Fungi bioaerosol CFU in 8 locations in selected schools.

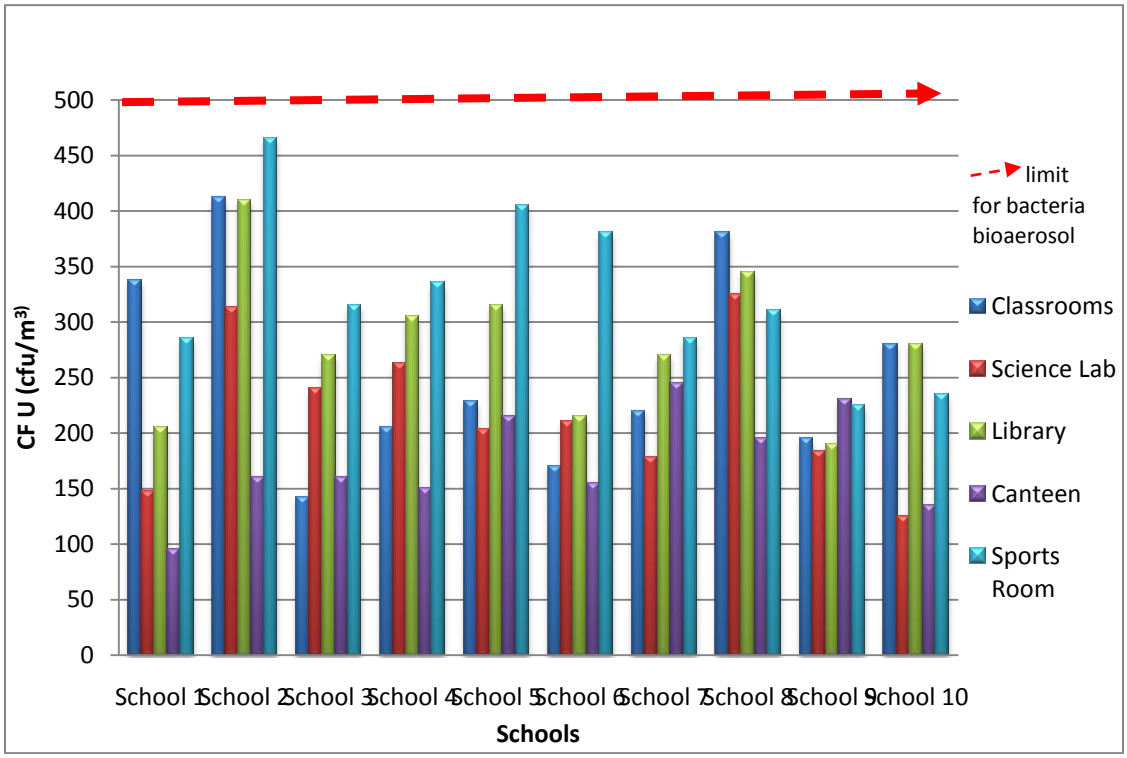

Fig. 4. Bacteria bioaerosol CFU in 8 locations in selected schools

Table 4
Occurrence of airborne fungi genus in 8 sampling locations of each school.

Genus	Occurrence range (%)	Number of location of airborne bacterial occurrence (N=8)									
		S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**
***Aspergillus* sp.**	75-100	8 (100)	6 (75)	7 (87.5)	7 (87.5)	6 (75)	8 (100)	6 (75)	7 (87.5)	7 (87.5)	6 (75)
***Penicillium* sp.**	37-75	3 (37.5)	6 (75)	6 (75)	5 (62.5)	4 (50)	3 (37.5)	6 (75)	5 (62.5)	5 (62.5)	4 (50)
***Acremonium* sp.**	0-25	0 (0)	0 (0)	2 (25)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (25)	1 (12.5)
***Culvularia* sp.**	0-38	0 (0)	0 (0)	0 (0)	0 (0)	1 (12.5)	0 (0)	3 (37.5)	0 (0)	1 (12.5)	0 (0)
***Cladosporium* sp.**	0-50	3 (37.5)	1 (12.5)	2 (25)	0 (0)	0 (0)	0 (0)	1 (12.5)	4 (50)	1 (12.5)	2 (25)
***Rhizopus* sp.**	0-75	0 (0)	1 (12.5)	0 (0)	4 (50)	3 (37.5)	6 (75)	0 (0)	1 (12.5)	0 (0)	1 (12.5)
***Geothrichum* sp.**	0-25	0 (0)	2 (25)	1 (12.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
***Verticillium* sp.**	0-25	1 (12.5)	2 (25)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (12.5)	0 (0)	0 (0)
***Paecillomyces* sp.**	0-25	0 (0)	0 (0)	2 (25)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
***Trichoderma* sp.**	0-13	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (12.5)	0 (0)
***Microsporum* sp.**	0-63	0 (0)	4 (50)	2 (25)	2 (25)	5 (62.5)	2 (25)	1 (12.5)	0 (0)	1 (12.5)	1 (12.5)
Unknown	0-38	2 (25)	3 (37.5)	0 (0)	0 (0)	0 (0)	0 (0)	3 (37.5)	0 (0)	0 (0)	0 (0)

Table 5
Occurrence of airborne bacteria genus in each school.

Genus/ species	Occurrence range (%)	Number of location of airborne bacterial occurrence (N=8)									
		S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**
		n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)
Staphylococcus aureus	62 -100	6 (75)	7 (87.5)	7 (87.5)	6 (75)	5 (62.5)	7 (87.5)	6 (75)	5 (62.5)	8 (100)	5 (62.5)
***Staphylococcus* sp.**	37 – 75	5 (62.5)	4 (50)	6 (75)	5 (62.5)	3 (37.5)	3 (37.5)	6 (75)	2 (25)	6 (75)	5 (62.5)
***Micrococcus* sp.**	12 – 38	2 (25)	1 (12.5)	3 (37.5)	0 (0)	1 (12.5)	1 (12.5)	0 (0)	2 (25)	0 (0)	3 (37.5)
***Streptococcus* sp.**	0 – 38	3 (37.5)	0 (0)	3 (37.5)	1 (12.5)	1 (12.5)	0 (0)	0 (0)	3 (37.5)	1 (12.5)	1 (12.5)
***Corynebacterium* sp.**	37 – 88	3 (37.5)	3 (37.5)	7 (87.5)	6 (75)	3 (37.5)	3 (37.5)	4 (50)	3 (37.5)	7 (87.5)	4 (50)
***Bacillus* sp.**	50 – 100	7 (87.5)	8 (100)	8 (100)	7 (87.5)	8 (100)	7 (87.5)	7 (87.5)	7 (87.5)	4 (50)	5 (62.5)
***Pseudomonas* sp.**	0 – 37.5	0 (0)	3 (37.5)	1 (12.5)	0 (0)	0 (0)	1 (12.5)	0 (0)	0 (0)	1 (12.5)	0 (0)
Unknown	0 – 12.5	0 (0)	0 (0)	1 (12.5)	1 (12.5)	1 (12.5)	1 (12.5)	0 (0)	1 (12.5)	0 (0)	1 (12.5)

The lowest occurrence was in School 8 (25%). Another Gram positive cocci identified was Micrococcus sp. which had relatively low range of occurrence (0-37.5%) compared to Staphylococcus aureus (62.5-100%). Highest occurrence was detected in School 3 and 10 (37.5%) while none was detected in School 4 and 7. Pseudomonas sp. had the lowest range of airborne bacterial occurrence (0-37.5%) whereby the highest occurrence was only in 1 school, School 2 and it was not found in 6 out of 10 schools.

School 1 to School 8 were urban schools while School 9 and 10 was suburban schools. Bacillus sp. was the highest occurring airborne bacteria in all 8 urban schools. However, the trend was not the same in suburban schools whereby Staphylococcus aureus was the highest in School 9 and Bacillus spp. was high but with other 2 genera of airborne bacteria having the same occurrence. In urban schools, the second highest was Staphylococcus aureus followed by Corynebacterium sp. In suburban schools, there was no specific trend of airborne bacterial occurrence (Table 5). Aspergillus sp., Penicillium sp. and Microsporum sp. was mainly found in the classroom locations while Culvularia sp. and Rhizopus sp. was found the most in science laboratory. Some fungi genus such as Verticillium sp., Paecillomyces sp. and Trichoderma sp. was not found in non-classroom locations such as library, canteen and sports room (Table 6). Staphylococcus aureus, Staphylococcus sp., Micrococcus sp., Corynebacterium sp., Bacillus sp. and Pseudomonas sp. was dominantly found in classrooms followed by the science lab. However, only Streptococcus sp. was found the most in science laboratory followed by library (Table 7). There was significantly higher concentration of airborne fungi in classroom compared to non classroom locations in School 1 ($p < 0.05$) (Table 8).

Table 6
Occurrence of fungi based on genus in each school's location.

Fungi (Genus)	**Occurrence based on genus**				
	Classrooms	**Science Lab**	**Library**	**Canteen**	**Sports room**
Aspergillus sp.	27	16	7	8	10
Penicillium sp.	18	13	6	5	6
Acremonium sp.	1	1	1	1	1
Culvularia sp.	1	3	1	0	0
Cladosporium sp.	4	2	1	5	2
Rhizopus sp.	3	4	3	2	4
Geothrichum sp.	0	0	1	1	1
Verticillium sp.	3	1	0	0	0
Paecillomyces sp.	2	0	0	0	0
Trichoderma sp.	0	1	0	0	0
Microsporum sp.	10	0	3	2	3

Table 7
Occurrence of bacteria based on genus in each school's location.

Fungi (Genus)	**Occurrence based on genus**				
	Classrooms	**Science Lab**	**Library**	**Canteen**	**Sports room**
Staphylococcus aureus	23	15	7	9	8
Staphylococcus sp.	15	9	7	7	7
Micrococcus sp.	5	2	4	2	0
Streptococcus sp.	1	6	4	1	1
Corynebacterium sp.	14	10	5	7	7
Bacillus sp.	23	17	9	9	10
Pseudomonas sp.	3	0	2	0	1

However, there were no significant difference in airborne fungi genus found in classrooms and non classroom location ($p > 0.05$) (Table 9). Significant statistical difference ($p < 0.05$) was observed between Streptococcus sp. in classrooms and non classroom locations in selected schools (Table 10). However, no statistical difference ($p > 0.05$) between other species of airborne bacteria found in classroom and non classroom locations in selected schools. Table 12 shows significant differences ($p < 0.001$) between airborne fungal CFU in urban (Mean Rank = 46.65) and

suburban (Mean Rank = 15.91) schools. No significant difference ($p > 0.05$) between airborne bacterial CFU in urban (Mean Rank = 42.81) and suburban schools was observed in the statistical test (Mean Rank = 31.25) (Table 13). No significant correlation was observed between airborne bacteria CFU in different location with environmental parameter. However, there was significant correlation ($r = 0.591$, $p < 0.001$) between airborne fungi bioaerosol CFU in classrooms with carbon dioxide level. There was no significant correlation between airborne fungi CFU in other locations with environmental parameter (Table 14).

Table 8
Difference between airborne fungi CFU in classrooms and non- classroom location in schools.

School	Location	(CFU) mean rank	X^2	*P*
School 1	Class	7.00	5.000	0.025*
	Non Class	3.00		
School 2	Class	5.33	0.556	0.456
	Non Class	4.00		
School 3	Class	3.00	1.800	0.180
	Non Class	5.40		
School 4	Class	4.50	0.000	1.000
	Non Class	4.50		
School 5	Class	4.00	0.200	0.655
	Non Class	4.80		
School 6	Class	2.33	3.756	0.053
	Non Class	5.80		
School 7	Class	4.50	0.000	1.000
	Non Class	4.50		
School 8	Class	6.00	1.800	0.180
	Non Class	3.60		
School 9	Class	2.67	2.721	0.099
	Non Class	5.60		
School 10	Class	2.33	3.756	0.053
	Non Class	5.80		

*significant at $p < 0.05$.

Table 9
Genera of airborne fungi in classroom and non classroom locations of the schools.

Species	Location (N=80)		χ^2	*p*
	Classrooms (n=30)	Non classrooms (n=50)		
Aspergillus sp.	27	41	0.941	0.332
Penicillium sp.	18	30	0.001	1.000
Acremonium sp.	1	4	0.697	0.404
Culvularia sp.	1	4	0.697	0.404
Cladosporium sp.	4	10	0.577	0.477
Rhizopus sp.	3	13	3.000	0.083
Geotrichum sp.	0	3	1.870	0.171
Microsporum sp.	10	8	3.231	0.072
Trichoderma sp.	0	1	0.608	0.436
Verticillium sp.	3	1	2.526	0.112
Paecillomyces sp.	2	0	3.419	0.064

Table 10
Genera of airborne bacteria in classroom and non classroom locations of the schools.

Species	Location (N=80)		χ^2	*P*
	Classrooms (n=30)	Non-Classrooms (n=50)		
Staphylococcus aureus	23	39	0.019	0.890
Staphylococcus sp.	15	30	0.762	0.383
Micrococcus sp.	5	8	0.006	0.938
Streptococcus sp.	1	12	5.884	0.015*
Corynebacterium sp.	14	29	0.969	0.325
Bacillus sp.	23	45	2.614	0.106
Pseudomonas sp.	3	3	0.432	0.511

*significant at $p < 0.05$.

Table 11
Difference between airborne bacteria CFU in classrooms and non- classroom location in schools.

School	Location	(CFU) mean rank	X^2	*p*
School 1	Class	6.00	1.800	0.180
	Non Class	3.60		
School 2	Class	5.67	1.089	0.297
	Non Class	3.80		
School 3	Class	2.00	5.000	0.025*
	Non Class	6.00		
School 4	Class	3.33	1.089	0.297
	Non Class	5.20		
School 5	Class	4.33	0.022	0.881
	Non Class	4.60		
School 6	Class	2.83	2.276	0.131
	Non Class	5.50		
School 7	Class	4.00	0.200	0.655
	Non Class	4.80		
School 8	Class	7.00	5.000	0.025*
	Non Class	3.00		
School 9	Class	4.00	0.200	0.655
	Non Class	4.80		
School 10	Class	6.33	2.689	0.101
	Non Class	3.40		

*significant at $p < 0.05$.

Table 12
Differences between airborne fungal CFU in urban and suburban schools.

Variable	Fungal CFU (mean rank)	Z	*P*
Urban	46.65	-4.734	0.001*
Suburban	15.91		

Table 13
Differences between airborne bacterial CFU in urban and suburban schools.

Variable	Bacteria CFU (mean rank)	Z	*P*
Urban	42.81	-1.781	0.075
Suburban	31.25		

Table 14
Correlation between bacteria and fungi bio-aerosol CFU and environmental parameter based on location.

Meteorological parameter	**Bacteria (CFU)**									
	Classroom (n=30)		**Science Lab (n=20)**		**Library (n=10)**		**Canteen (n=10)**		**Sports Room (n=10)**	
	r	***p***	**R**	***P***	**r**	***p***	**R**	***p***	**r**	***p***
Temperature (°C)	-0.024	0.889	-0.207	0.381	0.128	0.725	0.407	0.243	0.158	0.663
Relative Humidity (%)	-0.099	0.602	0.422	0.064	-0.085	0.815	-0.395	0.258	0.152	0.675
Carbon dioxide (ppm)	0.344	0.062	0.157	0.508	-0.097	0.789	-0.225	0.532	0.529	0.116
	Fungi (CFU)									
Temperature (°C)	-0.193	0.306	-0.012	0.96	0.03	0.934	0.304	0.393	0.152	0.675
Relative Humidity (%)	0.303	0.103	0.196	0.407	0.2	0.58	-0.359	0.309	0.006	0.987
Carbon dioxide (ppm)	0.591	0.001*	0.113	0.634	0.164	0.651	-0.213	0.555	0.164	0.65

*Significant at $p < 0.05$.

4. Discussion

Highest concentration of bioaerosol fungi was in sports room. Airborne fungi concentration in an indoor environment depends on the general hygiene of the place, the occupancy and the use of the place (Medrela-Kuder, 2003). Walk-through inspection revealed clearly that sports room were not cleaned frequently. School children enter the room to take equipments in and out of the room. Most of the time, the sports equipments were not cleaned before placing them back and the sports room remained closed at all times.

The second highest frequent concentration of airborne fungi was in the library. Six out of eight schools had high concentration of fungi in the library. In most selected schools, children use library during physical education theory lessons, reading lessons, literature, during recess and after school revision and also for moral education. It was estimated that a child spends an average of at least 6 hours in the library in a week. Libraries in selected schools had many suitable reservoirs such as settees, carpet, plants in pots, curtain and fabricated attention board which can enhance the growth of airborne fungi. Apart from that, used for the purpose of decoration in the library can be a contributing source towards airborne fungi concentrations (Adhikari et al., 2004).

In suburban schools, highest concentration of airborne fungi was observed in the library and canteen. A walkthrough inspection took place and observation revealed that plants were kept for decoration purpose inside and out of the library in school 9 which could be a cause for high concentration of fungi apart from the carpet, curtains and settees. The canteen in School 10 was an open place with the school field located within 20 meters from the canteen. The field could be the source of high airborne fungi concentration especially during physical education lesson. It also depends on the wind movement direction. Although the airborne fungi concentration is high within the suburban school, it is lower than the airborne fungi concentration in urban schools.

Significant difference was noticed between classrooms and non classroom locations in School 1 with higher concentration of fungal CFU in the classrooms. This result was expected as obvious signs of black spores were noticed on the ceiling during the walkthrough inspection. Some part of the classrooms was not fully covered with ceiling and left exposed. There were mould stains on the wall of a classroom in the school. However, the classrooms were well maintained and clean during the sampling.

Non-consistence in the concentration of airborne fungi in classrooms and non classroom location can be justified with sampling time. Sampling in School 3 took place during exam week whereby children were in exam hall than the classrooms. Two out of three classrooms were with less than five occupants. During exam week, most of the other locations were kept closed such as the library, science laboratory and sports equipment room. This could have caused high concentration of airborne bacteria in non classroom locations thus creating a significant difference.

Presence of airborne bacteria was higher in urban than suburban area schools. This is most probably due to the surrounding human activities and land use near the selected schools. Schools in urban area were located close to busy main roads and industrial areas while schools in suburban area were not as exposed as urban schools. Significant difference was observed between Bacillus sp. in urban and suburban schools. Bacillus sp. is a gram positive spore forming bacteria. Due to its spore forming capabilities, this genus of bacteria will be airborne once cleaning or human activities takes place in a particular place. Probability of higher human activities in urban schools can be taken into consideration as during sampling, it was towards year end and in most schools, children were having activities such as board game competitions and text book returning or class segregation. Suburban school sampling was done during middle of the schooling semester.

Apart from that, age and maintenance of the buildings in suburban schools can be a factor for higher concentration of Bacillus sp. in urban schools (Gorny et al., 1999). School 9 was built in 1995 and was repainted in early 2011 while form 2 classrooms, science laboratory and library in School 10 were in a new building which was built in 2001. Both School 9 and 10 were suburban schools. There were no signs of water leakage in sampling locations in suburban schools. High concentration of Bacillus spp. in a building indicated previous history of water damage or lack of maintenance (Peltola et al., 2001).

Gram negative bacteria become inactive in relative humidity higher than 85% (Robine et al., 2000). Schools in urban areas showed measurement of high relative humidity (73 to 88.1%) which can reduce the concentration of bacteria in urban school indoor air. Relative humidity in suburban schools were lower therefore does not lead to inactivation of bacteria (65.4 to 81.6%). Non significance between airborne bacteria in urban and suburban schools can be caused by relative humidity.

The optimal condition for fungal growth is above 70% of relative humidity and temperature between 30 to 40°C (Burge et al., 1995). Relative humidity measured in most urban area schools was within the range of optimal growth condition. However, relatively low humidity was measured in suburban school (Table 6). Higher mean rank for airborne fungi in urban schools suggested that surrounding land use and human activities that varies between urban and suburban school also plays a role in the abundance of these airborne microorganisms.

There was no optimal temperature or relative humidity for airborne bacterial growth as it may vary with species (Tang, 2009). Airborne fungi concentration tends to be influenced by seasonal variation or seasonal changes in the indoor climate factors (Karra & Katsivela., 2007). Similar to airborne bacteria, airborne fungi also have different optimal temperature and relative humidity based on species (Tang, 2009).

Airborne fungi CFU only in classrooms were tested for correlation with carbon dioxide, significance was noted ($p < 0.05$). High occupancy level in classrooms compared to other locations of the school may indicate significance between airborne fungi CFU and carbon dioxide level. Children spend more time in the classrooms compared to all the other locations during schooling session.

Airborne fungi CFU were tested with temperature, relative humidity and carbon dioxide and there were significant positive correlation between airborne fungi and carbon dioxide ($p < 0.05$). Previous literature shows that carbon dioxide had been fungal hyphae growth stimulator (Becard et al., 1989). Carbon sources from carbon dioxide have been an essential growth factor for fungi. Therefore, carbon dioxide level influences airborne fungi growth in indoor environment.

About 6 different species of airborne bacteria found and 5 species were Gram Positive Bacteria (GPB) while only Pseudomonas sp. was Gram Negative Bacteria (GNB). This finding is consistent with other findings by Fang et al. (2007) and Husna et al. (2011). The outer coat of GPB is a thick peptidoglican layer while GNB is surrounded by thin lipopolysaccharide coat. The thick layer of GPB's outer layer allows water to be retained inside the cell thus is not effected by environmental factors especially humidity (Nikiyan et al., 2009). This explains the dominance of GPB that were isolated in this study. According to Koneman et al. (1997), GNP is found in abundance in the environment and in human or animal body sites. Its wide range of habitat allows it to be transferred easily during human activities around the sampling area.

Common airborne fungi found in the environment worldwide were Aspergillus and Penicillium species (Hardin et al., 2003). Factors such as moisture content, inadequate ventilation, relative humidity and temperature influence the concentration of fungal spores. This study took place in selected schools which are divided into different location that can serve as an ideal location for airborne fungal reservoir. This has caused the level of airborne fungi to exceed in 8 out of 10 schools. Adding on to that, the occupancy level has further facilitated the growth of airborne fungal concentration. However, the significance found between concentration of carbon dioxide and airborne fungi shows that possibilities of airborne fungi spores to be blown in to the school building can be overruled. Natural ventilation and fan units can be a factor for increasing amount of spores indoor (Burge et al., 2000). Spores can withstand unfavourable environmental condition as it can remain dormant until environmental condition becomes favourable. This also explains the high concentration of fungi in the selected schools.

Aspergillus sp. is characterized as spore bearing structure called conidiophores. The production of Aspergillus sp. spores is often extraordinary which makes it as one of the common fungi on earth (Klich, M. A., 2009). These airborne fungi are potentially life threatening and linked with hypersensitivity reactions such as rhinitis, sinusitis and asthma (Tang et al., 2013). Aspergillus sp. was commonly found in the classroom locations followed by science laboratory. Classrooms were built with door and window panels in both left and right hand side of the classrooms and science lab. Therefore, it is easier for spores to enter and reside in reservoirs in the locations. Library and sports room windows and sometimes doors are commonly left closed with fewer occupants and canteen is an open space that looks like shed. Classrooms and science laboratories are most occupied location compared to canteen, library and sports room. The elevated level of carbon dioxide facilitates the growth of airborne fungi (Becard et al., 1989). Penicillium sp. has similarities with Aspergillus sp. whereby the spores are same in size and shape. These two genera can be distinguished by its spore bearing structure. Penicillium sp. also has similar effects as Aspergillus sp. on human health. Both genera can produce mycotoxin which has adverse health effects to human especially immunocompromised ones (Tang et al., 2013).

Microsporum sp. commonly found in indoor and outdoor such as soil (geophilic species), on animals (zoophilic species) and human (anthropophilic species). They are dermatophytes which cause dermatological infections in cats, dogs (zoophilic) and humans (Garcia-Cruz et al., 2012). This explains why concentration of

Microsporum sp. was highest in classroom. This could also be supported by the probability that the classroom occupants might also have pets such as cats and dogs. Contrast to the findings of Aspergillus sp. and Penicillium sp., none were found in the science laboratory. Occupants of the science lab are students in classes that have experiments which are about an hour or two in a week depends on how many periods has been assigned to the class. Other than experiment periods, the science lab will remain closed. The possibility of less occupied and the disinfectants used in science lab such as ethanol may be cause the absence of Microsporum sp. in science lab.

Staphylococcus sp. is GPB that looks like clusters of grapes. They are normal flora commonly found in the nasal cavity and skin of humans and categorized as opportunistic pathogens that can produce serious infections (Murai et al., 1995). Adaptation to a wide range of temperature (6.5-50°C) and found commonly in warm moist places shows why this airborne bacteria is found in high concentration in this study (William et al., 2004). Body temperature of human carrier is within the range and tropical weather allows Staphylococcus sp. to grow. High concentration of Staphyloccous sp. was detected in classroom and science laboratory because environmental parameter (temperature and relative humidity) was within the suitable range of growth with occupants who are possible human carriers of this airborne bacterium.

Bacillus sp. is accounted as the second highest airborne bacteria found in the selected schools mostly in the classroom and science lab. Bacillus sp. belongs to the group of gram positive which are able to produce physically and chemically resistant endospores (McKillip., 2000). This explains why Bacillus sp. was found in abundance in selected schools. Bacillus sp. produces exotoxin that causes skin edema, anthrax, food poisoning and more (Mead et al., 1999; Kotiranta et al., 2000).

Corynebacterium sp. is the third highest concentrated airborne bacteria found mainly in the classroom and also science laboratory. Similar to Staphylococcus sp. and Bacillus sp., Corynebacterium sp. is also GNP. They are non-sporing, non-motile and normal flora. Due to an increase in the number of immunocompromised patients, Corynebacterium sp. is taken as opportunistic pathogen that can be found in respiratory tract, wound, urinary tract and causes diphtheria (Shukla et al., 2001).

High concentration of Staphylococcus sp., Bacillus sp. and Corynebacterium sp. in classroom and science laboratory is influenced by the occupancy of the location as human are the main carrier. Suitable environmental parameter such as temperature and relative humidity has facilitated the growth of these airborne bacteria in these locations. Furthermore, ventilation too plays a role in circulating these airborne microorganisms. However, it cannot be denied that the library, canteen and sports room also has the same factors but the occupancy level and air ventilation may influence the concentration.

5. Conclusion

Airborne bacteria did not show any correlation with the environmental parameters measured within school locations and within schools in urban and suburban areas. However, airborne fungi showed significant correlation for carbon dioxide in classrooms. Carbon dioxide was the indicator of air ventilation and occupancy in an indoor air. High occupancy can lead to higher level of carbon dioxide. High level of carbon dioxide causes increased growth of airborne fungi in classrooms. This suggests that when limited occupants are in the classroom, the growth pattern of airborne fungi may slow down.

Concentration of airborne fungi and bacteria was higher in urban than suburban schools. Apart from the fact that urban settlement does not have greeneries surrounded, other factors such as school age, maintenance, occupancy level and surrounding land use might contribute to the concentration of these microbial bioaerosol.

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