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Assessment of indoor air micro-flora in selected schools

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Abstract. Quantification of viable forms of microbial community (bacteria and fungi) using culture-dependent methods was done in order to characterize the indoor air quality (IAQ). Role of those factors, which may influence the concentration of viable counts of bacteria and fungi, like ventilation, occupancy, outdoor concentration and environmental parameters (temperature and relative humidity) were also determined. Volumetric-infiltration sampling technique was employed to collect air samples both inside and outside the schools. As regard of measurements of airborne viable culturable microflora of schools during one academic year, the level of TVMCs in school buildings was ranged between 803-5368 cfu/m^3. Viable counts of bacteria (VBCs) were constituted 63.7% of the mean total viable microbial counts where as viable counts of fungi (VFCs) formed 36.3% of the total. Mean a total viable microbial count (TVMCs) in three schools was 2491 cfu/m^3. Outdoor level of TVMCs was varied from 736-5855 cfu/m^3. Maximum and minimum VBCs were 3678-286 cfu/m^3 respectively. Culturable fungal counts were ranged from 268-2089 cfu/m^3 in three schools. Significant positive correlation ($p < 0.01$) was indicated that indoor concentration of viable community reliant upon outdoor concentration. Temperature seemed to have a large effect ($p < 0.05$, $p < 0.01$) on the concentration of viable culturable microbial community rather than relative humidity. Consistent with the analysis and findings, the concentration of viable cultural counts of bacteria and fungi found indoors, were of several orders of magnitude, depending upon the potential of local, spatial and temporal factors, IO ratio appeared as a crucial indicator to identify the source of microbial contaminants.

Keywords: colony forming units (CFUs); indoor air quality (IAQ); indoor-outdoor (IO) ratio; relative humidity (Rh); temperature; viable counts of bacteria and fungi (VBCs and VFCs)

1. Introduction

Indoor air is the most vital environment with respect to our health besides being a dominant source of contaminants (Sundell 2004). It contains a complex mixture of biological and non-biological particles incorporated with dust. Among these, biological contaminants confer a considerable meaning in the elevation of indoor air pollution as they can be pathogenic or may cause allergic reactions, trigger the respiratory problems after inhalation and cause adverse health effects (Brooks *et al*. 2005, Kelman *et al*. 2004, Karvala *et al*. 2008, Lingnell *et al*. 2007).

Viable microbial frictions of biological contaminants are metabolically active organisms with the potential to reproduce. These contaminants may be culturable and non-culturable (Jensen and Schafer 1998). Assessment of microbial contaminants in the form of bacteria and fungi from

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indoor environment is always a challenging and worthwhile subject of great concern (Matkovic *et al*. 2006, Rogers 2003, Stetzenbach *et al*. 2004). Detection and quantification of culture based microbial analysis is useful in order to assess the exposure of variety of microbial contaminants in the occupational environment (Lucette *et al*. 2005). Intensity of microbial contaminants and exposure assessment have been studied extensively in various environment like rural environment (Adhikari *et al*. 2004a, 2004b), poultry houses and egg processing facilities (Bakutis *et al*. 2004, Northcutt *et al*. 2004), hospital environment (Augustowska and Dutkiewicz 2006), industrial and official environment (Kalogerakis *et al*. 2005, Park *et al*. 2006), enumeration of residential and house dust (Giovannangelo *et al* 2007, Niemeier *et al* 2006, Ping Ren *et al* 1999).

Schools are the facilities for educational pursuits. Hence, apart from the residential environment, school is the second most important indoor environment in evaluating the quality of indoor air and health components of occupants (Meyer *et al*. 2005, Smedge *et al*. 1997, Santilli 2002, Santilli and Rockwell 2003, Godwin and Batterman 2007). In schools, children spend a reasonable portion of their time. Moreover, children are considered potentially more susceptible to environmental exposure than adults (Aprea *et al*. 2000, Ebbehoj *et al*. 2005, Patovirta *et al*. 2004).

In India, the concern about the condition of indoor air quality is an evolving issue of investigations since sensitivity of exposure to multiple contaminants has become more widely known. Taking into consideration, present study was designed to evaluate the concentration and variability of airborne viable-culturable microbial community (Bacteria and Fungi) in classroom environment of schools so as to assess IAQ. Acquired data were compared with the other international standards and guidelines accessible for the indoor air microbial load.

2. Methodology

2.1 Study area

Delhi, the capital of India is a fastest growing metro city. The city limits have virtually been divided into zones by Delhi Municipal Corporation as - North, South, East, West and Central. Schools, located in North Delhi area have been selected for IAQ investigations.

2.2 Characteristics of classrooms

Prior to air sampling, classroom characteristics *viz.* type of ventilation, number of installed fans and exhaust system, floor area of selected classroom, ceiling height, volume, furniture status, walls, cleanliness, number of students, average occupancy daily and on the day of sampling, visible appearance of microbial growth, if any, were examined and recorded during the air sampling session (Table 1). All schools were having the status of senior secondary level and usually, students from ages 11 to 18 years were studying there.

2.2.1 School 1 (S1)

Building was constructed around 70 years ago. Visible symptoms of small black mold patches on the walls and enormous deposition of dust on the various surfaces were noticed during investigation. Terrible damp smell was also experienced there in teacher’s room.

2.2.2 School 2 (S2)

Table 1 Characteristics of classrooms in three schools

Characteristics	S1	S2	S3
Name	DAV School Daryaganj	Govt. SS School Dhakka	AS School Dayyaganj
Location	North Delhi	North Delhi	North Delhi
No. of Floors	Two	Three	Two
Floors investigated	GF*	GF & TF**	GF & FF***
Ventilation	Natural	Natural	Natural
Sun Light	Too dim to moderate	Moderate to bright	Too dim to moderate
Walls	Too dirty	Dirty	Dirty
Dampness	Yes	No	No
Odor	Yes	Yes	Yes
Cleaning	Daily but inadequately	Daily but inadequately	Moderately
• *Sweeping*	Rarely	Sometimes	Sometimes
• *Moping*	No	Rarely	Daily
• *Moping with pesticide*	No	No	Rarely/partially
Dustbins	Uncovered	Uncovered	Uncovered
Dust deposited on surface			
• *Horizontal*	Yes	Yes	Yes
• *Vertical*	Yes	Yes	Yes
Mechanical exhaust	No	No	No
Cooling system	Fans	Fans	Fans

*GF = Ground floor; **FF = First floor; ***TF = Third floor

Inside conditions of the school were more or less similar to School 1. No visible sign of mold growth was seen in the classrooms, though sometimes bad odor was experienced.

2.2.3 School 3 (S3)

As per the records available, the construction of building was done in 1910 hence the architecture of the building has incredibly old type. The schools surrounded by enormous greenery including big trees, shrubs and ornamental plants all around the building. Classroom conditions were seemed to be little better than two schools. Deposition of dust was also pronounced at some places.

2.3 Air sampling

Air sampling, for detection and isolation of viable counts of bacteria and fungi was carried out for a period of ten months as academic session commences from July to April. Twice in a month air sampling both inside and outside the three schools were carried out in order to characterize the IAQ. Number of sampling locations in each school and sampling protocol was described in Table 2. Air sampling for presence of culturable viable counts of bacteria and fungi was conducted at respiratory height (at 1.2 meter) for 25 minutes using air samplers APM 823 (Envirotech Pvt Ltd., India), which was operating at flow rate of 28.5 liters per minute (LPM) and sucked through poly-tetra-flouro-ethylene (PTFE) membranes (47 mm diameter, 0.4 μm pore size).

Table 2 Details of sampling protocol

Sampling	S*1	S2	S3
Type	Stationary	Stationary	Stationary
Technique	Volumetric/infiltration	Volumetric/infiltration	Volumetric/infiltration
Sampling Stations			
Indoor	2	2	2
Outdoor	1	1	1
Frequency			
Indoor	40	40	80
Outdoor	20	20	20

*S = School/s

2.4 Suspension of air samples

Each PTFE membrane was soaked in 10 ml of sterile normal saline and vigorously shaked using vortex mixture for 5 min to prepare a suspension of the trapped particles. From this suspension, 100 μl was used for spread plate culture on duplicate Petri dishes (10 cm diameter) containing respective culture media for isolation of viable counts of Bacteria and Fungi.

2.5 Enumeration of airborne viable counts of bacteria (VBCs)

Nutrient agar (NA) (Hi media laboratories) medium plates were used for enumeration and quantification of airborne VBCs. NA plates were incubated in B.O.D. incubator at 37° ± 1°c for 24 hours.

2.6 Quantification of airborne viable counts of fungi (VFCs)

Same suspension of air sample was also used for isolation of fungi. Rose Bengal Streptomycin Agar (RBA) (Hi media laboratories) medium was used for isolation of viable counts of fungi. RBA plates were incubated at 28 ± 2°C for 5 days.

Various fungal and bacterial colonies in each sample were counted and calculated by direct colony count or using colony counter and expressed as colony forming unit. Data was reported as colony forming unit (cfu) per cubic meter of air on the basis of the total air sampled on each filter membrane. Following formula was used for calculating CFUs

$$cfu/\mathrm{m}^3 = \text{No.of colonies in } 0.1\,\mathrm{ml}/0.1\,\mathrm{ml} \times 10\,\mathrm{ml}/\text{volume of sample dair}(\mathrm{m}^3)$$

2.7 Quantification of total viable microbial counts (TVMCs)

Quantification of total viable microorganisms in indoor and outdoor air was acquired summing the total number of viable counts of bacteria plus total number of viable counts of fungi obtained from above steps.

2.8 Micrometeorological measurements

During the study period microclimate parameters, temperature (°C), and relative humidity (Rh%) of indoor and outdoor air were measured using Thermo hygrometer (Easl Scientific Industries India) in order to assess the influence of meteorological variations on the concentration of viable counts of bacteria and fungi.

2.9 Statistical analysis

One academic year data (10 month data) was presented here. Monthly samples were taken during July 2005 to April 2006 from inside and outside the schools. Statistical analysis of data was done in order to evaluate the monthly dynamics of viable counts of bacteria and fungi found in air of inside and outside the school. Data was analyzed by column statistics using prism for windows VISTA. Descriptive statistics included mean, minimum, maximum, standard deviation, one sample *t' test*, Wilcoxon signed rank test, were employed for the data analysis. One way *ANOVA* was applied to study the significant difference in concentration of viable culturable counts of bacteria and fungi. Paired *t-test* was selected because the samples were collected parallel from indoor and outdoor air. Spearman's test for correlation-coefficients was established to determine the statistical correlation of indoor and outdoor concentrations for airborne viable bacterial and fungal counts. Spearman correlation was also calculated for comparing the relationship of meteorological factors (temperature and relative humidity) on VBCs and VFCs. Indoor to outdoor ratio (IO ratio) of viable counts of bacteria and fungi of each month was ascertained the relationship between outdoor concentration of viable counts of bacteria and fungi.

3. Results

Quantitative estimation of air, which was accumulated in the form of dust on PTFE membrane, was carried out in order to enumerate the airborne VBCs and VFCs of indoor and outdoor air of schools. Table 2 shows the details of air sampling protocol whereas description of indoor sites was given in Table 1. The results of indoor air and comparison of the results with the viable counts of Bacteria and Fungi of outdoor air as well as meteorological factors are presented here. The graphics (arithmetic mean) and tabular forms are allowed a comprehensive analysis of results of air sampling carried out inside and outside the schools. Descriptive statistics of TVMCs and magnitude of VBCs and VFCs of each month were presented in tabular form (Table 3).

3.1 Total viable microbial counts (TVMCs)

TVMCs were accounted for sum of mean of viable counts of bacteria and fungi of each month and yielded as summing up the viable counts of bacteria of each month with viable counts of fungi of the corresponding month. Figs. 1 and 2 illustrate the inside and outside mean TVMCs according to their collection period in three schools investigated in this study. It was ranged between < 1000 - > 5000 cfu/m^3 inside the classrooms all three schools (Fig. 1). The incidence of total viable counts of microbial community in three schools was heterogeneous correspond to various months. Range of TVMCs was inside and outside of three schools was given in Table 3.

Table 3 Descriptive statistics of total viable counts measured inside and outside of the three schools

Analysis	S*1I**	S2I	S3I	S1O***	S2O	S3O
N	40	40	80	20	20	20
TVMCs						
AM[1]	3033	2753	1691	3022	3073	1823
SD[2]	1391	1567	597.1	1333	1706	657.4
Range	1405-5577	689-4963	504-2646	1286-5556	602-5980	946-2834
GM[3]	2752	2257	1574	2738	2512	1708
[a]*P* value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Significant (alpha = 0.05)?	Yes	Yes	Yes	Yes	Yes	Yes
[b]*P* value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Significant (alpha = 0.05)?	Yes	Yes	Yes	Yes	Yes	Yes
Magnitude (%)						
Bacteria	61.13	70.25	57.86	66.15	73.71	56.28
Fungi	38.87	29.73	42.11	33.82	26.31	43.75

* S = School/s; ** I = Indoor; *** O = Outdoor
[1]AM: Arithmetic mean; [2]SD: Standard deviation; [3]GM: Geometric mean
[a]One sample *t' test*; [b]Wilcox on signed rank test

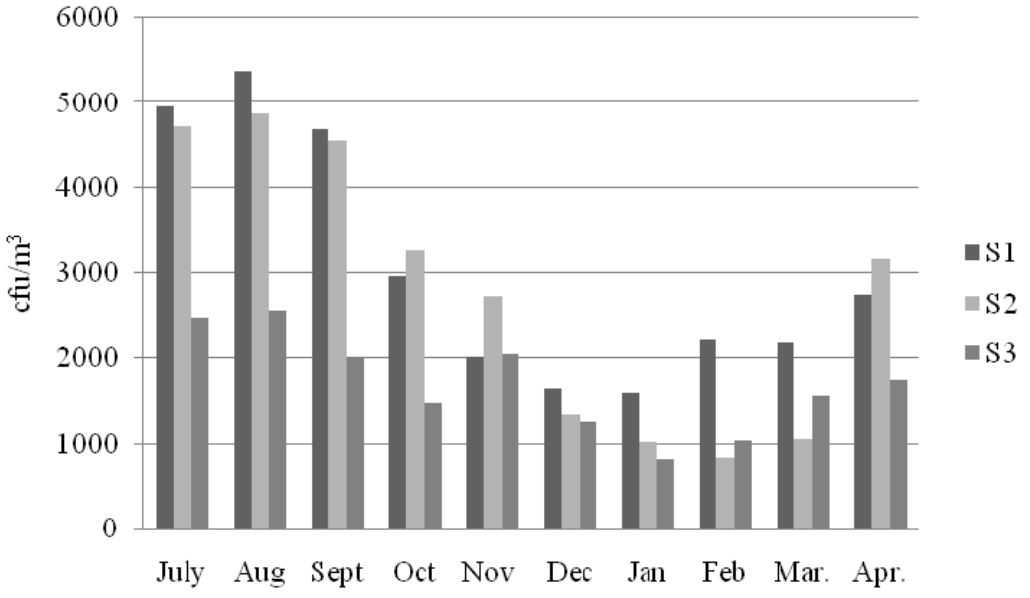

Fig. 1 Total microbial viable counts (TVMCs) inside the three schools

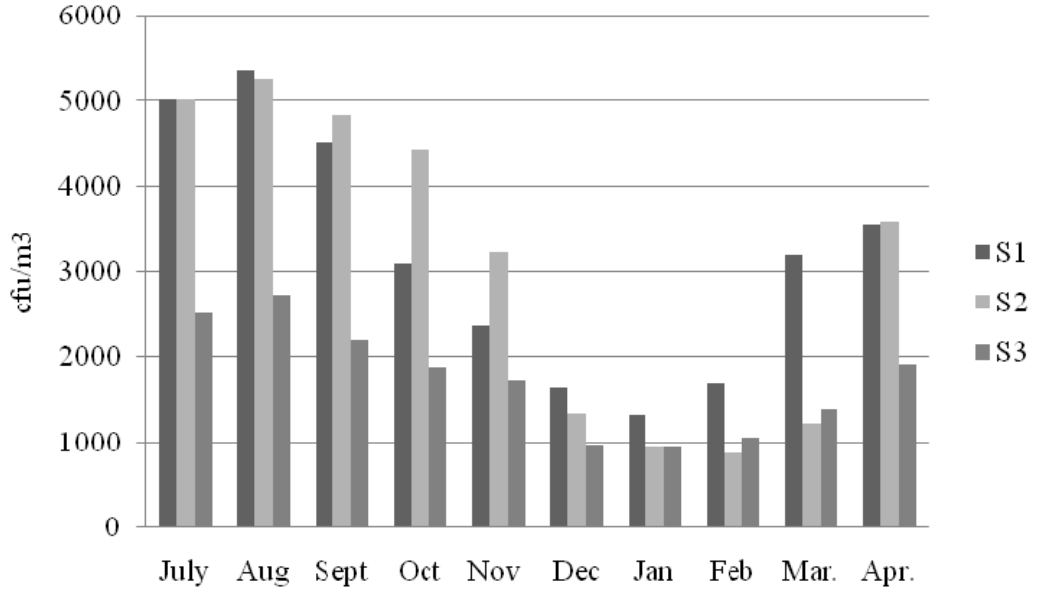

Fig. 2 Total microbial viable counts (TVMCs) outside the three schools

Number of TVMCs was relatively higher at the School 1 followed by School 2 ($P < 0.0001$). On five instances, its counts in the air of three schools were lying on < 1000 cfu/m^3. Fig. 1 illustrates the marked difference in concentration of TVMCs found in School 3 which was observed lower than other two schools ($P < 0.0001$).

As regard of monthly analysis of TVMCs of three schools, it was apparent that total viable counts in School 1 were constantly measured at above the 1000 cfu/m^3. Mean TVMCs per month was 2985 cfu/m^3 (± 1422). The indoor concentration of TVMCs in School 1 was between 1405 to 5368 cfu/m^3. Mean TVMCs at School 2 was observed 2697 cfu/m^3 (± 1607). It was within the range of 689 to 4963 cfu/m^3. Mean concentration per month in the air of School 3 was 1518 cfu/m^3 (± 608).

Fig. 2 reveals the evaluation of TVMCs found outside three schools investigated in this study. Only four number of air samples were shown the concentration below than 1000 cfu/^{3}. Concentration of TVMCs was exceeded above the 5000 cfu/m^3 twice. Mean concentration was higher in outside air of School 1 than in other two schools ($p < 0.001$). The mean TVMCs were 3177 cfu/m^3 (± 1445.92) all through the air sampling at outside the School 1. Sampling and analysis of air outside the School 2 was shown that the mean TVMCs 3083 cfu/m^3 (± 1814.33) with great variation (736-5563 cfu/m^3). The results indicate that mean TVMCs of outside of School 3 was 1738 cfu/m^3 (± 633.5). At this place it was measured in the range of 854-2850 cfu/m^3.

One way ANOVA reveals the significant difference among the concentration of TVMCs in the air of three schools ($P < 0.0001$). Paired *t-test* reveals no significant differences between concentrations of TVMCs found in indoor and outdoor air of three schools. Table 3 revealed that TVMCs were comprised a large portion of bacteria (50-70% of TVMCs) rather than fungi (> 26 - < 50%).

3.2 Viable bacterial counts (VBCs)

The indoor concentrations of VBCs of each month in all three schools were presented in Fig. 3. The magnitude (%) of the total number of VBCs obtained over the course of the study from the indoor and outdoor was given in Table 3. Mean monthly concentrations of VBCs in the air of examined schools were 1589 cfu/m^3. Eight number of samples were found to have concentration of VBCs below than 1000 cfu/m^3 whereas four number of air samples were come into view to have concentration of VBCs > 3000 cfu/m^3 in three schools.

Mean concentration of VBCs in each month in indoor air of School 1 was 1854 cfu/m^3 (± 913.47) with the highest counts 3483 cfu/m^3 in the August followed by July (2900 cfu/m^3) and the lowest counts was measured in winter months i.e., January (948 cfu/m^3) and December (1070 cfu/m^3). At this place VBCs was ranged between 848-3569 cfu/m^3. Average number of VBCs per month outside School 1 was found to be 1999 cfu/m^3 (± 904.74). VBCs were evidenced significantly utmost in the month of August (3477 cfu/m^3) and minimum in month of January (687 cfu/m^3) (Fig. 5). The concentration of VBCs at this place was on higher range in July (2964 cfu/m^3), September (2683 cfu/m^3), October (2216 cfu/m^3), March (2147 cfu/m^3), April (2111 cfu/m^3).

Monthly concentration of VBCs in air of School 2 was ranged between 423-3490 cfu/m^3 with the peak number of counts in September (3604 cfu/m^3) followed by August (3326 cfu/m^3). In School 2 VBCs was ranged between 514-3901 cfu/m^3 with average number of counts in terms of colony forming unit per cubic air were found 2264.6 (± 1438.56) (Fig. 2). Highest counts were observed in September (3901 cfu/m^3), followed by August (3733 cfu/m^3) and July (3524 cfu/m^3) where lowest counts were found in month of January (514 cfu/m^3) followed by February (586 cfu/m^3) and March (707 cfu/m^3).

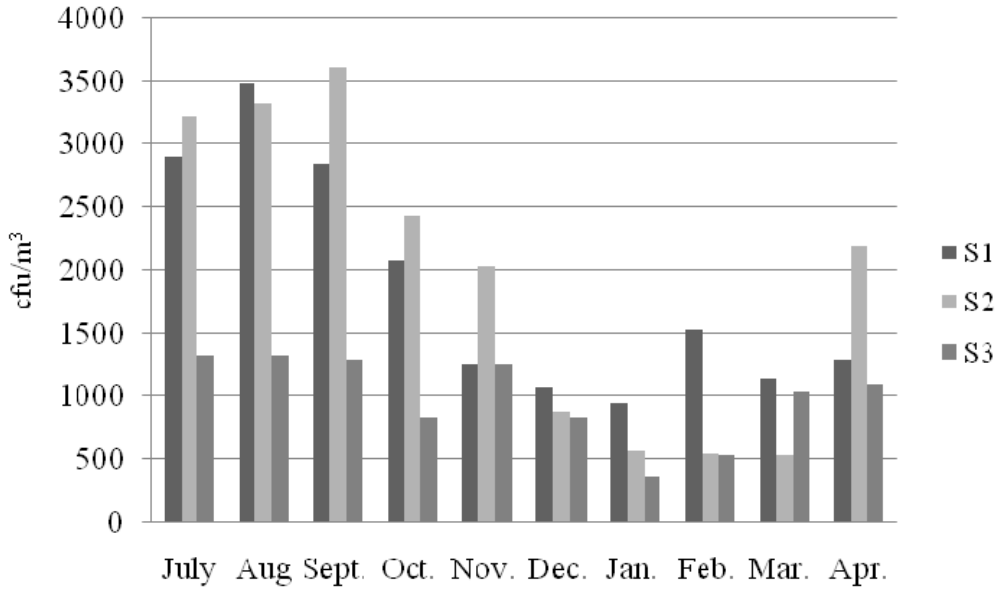

Fig. 3 Viable counts of bacteria (VBCs) measured inside the three schools

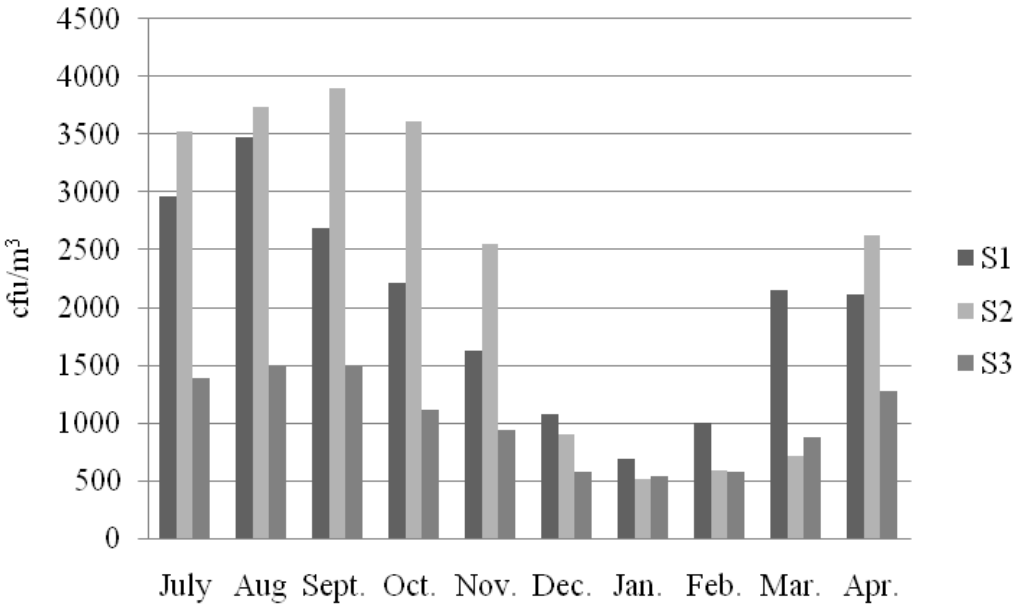

Fig. 4 Viable counts of bacteria (VBCs) measured outside the three schools

The indoor concentration of VBCs throughout the air sampling in School 3 was ranged between 286c to 1456 cfu/m^3 with the mean counts 978 cfu/m^3 (± 349.50) in each month. Monthly analysis of VBCs outside air of School 3 illustrates that counts were utmost in the month of July and August (1326 cfu/m^3 and 1325 cfu/m^3). The lowest number of VBCs was measured in the month of January (371 cfu/m^3). All through the ten month sampling period outside the School 3, VBCs was found on lower side, as average counts of bacteria in terms of cfu/m^3 were 1025 (± 382.39).

One way ANOVA was established the significant difference between the concentrations of VBCs in and outside air of all three schools ($P < 0.0001$). Paired *t* test was shown no significant differences between concentrations of VBCs found in indoor and outdoor air of three schools.

3.3 Viable fungal counts (VFCs)

Mean concentrations of total VFCs in and outdoor air in each month were presented in Figs. 5 and 6. Graphical analysis elucidated that nine number of samples were found to have VFCs concentration > 1000 cfu/m^3, whereas on four times, concentration of VFCs was measured > 1500 cfu/m^3.

The mean concentration of viable counts of fungi in indoor air of School 1 was utmost in the July (2055 cfu/m^3), corresponding to lowest counts measured in month of December (562 cfu/m^3). Mean VFCs throughout the sampling period at this place was 1178 cfu/m^3 (± 576.71). Fungal counts in per cubic meter of outdoor air throughout the air sampling at School 1 were varied from 461 to 1852 cfu/m^3 with average counts 1021 cfu/m^3 (± 443.45) in each month.

The concentrations of indoor VFCs in School 2 were in the range of 290-1540 cfu/m^3. Mean viable counts of fungi per month inside the School 2 were 818.46 cfu/m^3 (± 431.90). Number of

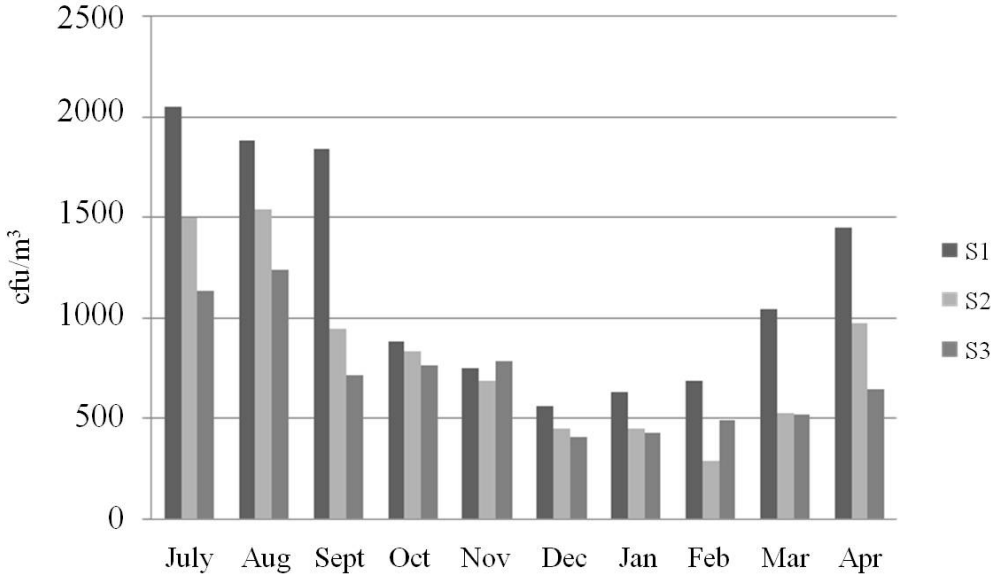

Fig. 5 Viable counts of fungi (VFCs) measured inside the three schools

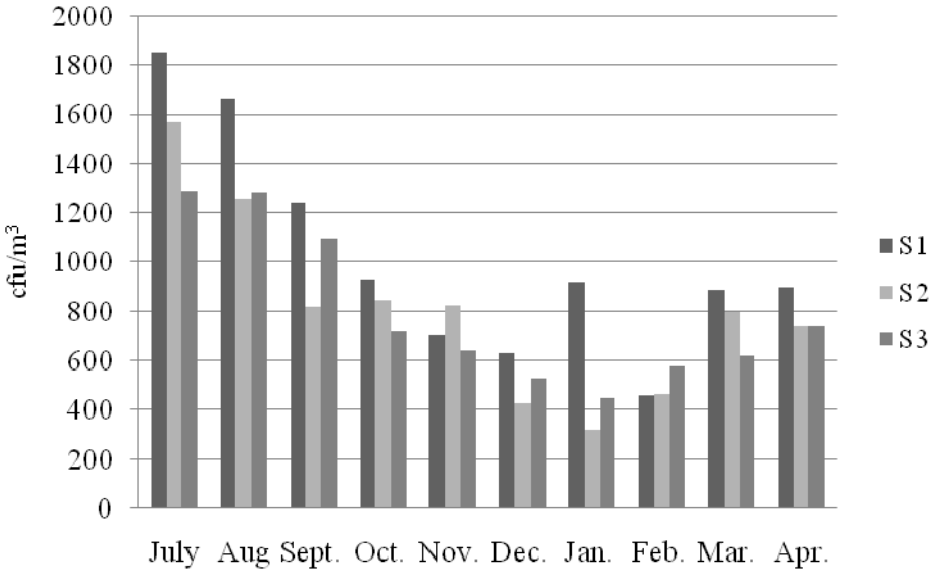

Fig. 6 Viable counts of fungi (VFCs) measured outside the three schools

VFCs outside the School 2 was ranged between 319-1573 cfu/m^3. The average number of counts during the entire air sampling session was 808.63 cfu/m^3 (± 379.34). Mean VFCs throughout the air sampling at this place were 712.13 cfu/m^3 (± 283.30), highest counts were in measured in the August (1236 cfu/m^3 p = 0.0017) followed by July (1134 cfu/m^3) whereas lowest counts were in the December (408 cfu/m^3), followed by January (430 cfu/m^3) and February (491 cfu/m^3).

The CFU counts outside the School 3 were ranged between 449-1292 cfu/m^3 with average counts per month was 797.6 cfu/m^3 (± 311.46). Lowest number of VFCs was measured at School 3 as compare to other two schools. One way ANOVA was established the significant difference between the concentrations of VFCs in and outside air of all three schools ($P < 0.0001$). No statistically significant difference was observed in paired *t* test between concentrations of VFCs of indoor and outdoor air of three schools.

3.4 Micro-meteorological parameters

Temperature (°C) and relative humidity (%) was recorded at each spot during the sampling. Monthly averages of temperature and relative humidity were recorded during sampling at each sampling locations is presented in Table 4.

3.5 Indoor to outdoor ratio (IO) for VBCs and VFCs

IO ratio is possible way to identify and locate the source of pollutants found in indoor environment. In order to examine the effect of concentration of viable counts of bacteria and fungi, measured outside and in the classrooms, an indoor to outdoor ratio of Bacteria and Fungi of each month has been calculated. Table 5 has revealed the statistical analysis of IO data. Figs. 7 and 8 show the mean IO ratio of bacteria and fungi in each month in three schools. The IO ratios for the Bacteria and Fungi ranging in 0.4 to 1.9 were determined.

IO ratio for VBCs in three schools was varied from 0.38 to 1.97. On nine times mean IO ratio for bacteria was calculated greater than one and on eight time it was measured close to 1 (1 to 0.9). The IO ratio for VBCs at School 1 was measured between 0.44-1.52. The mean IO ratio of each month at this place was calculated, > 1 or close to 1 in July (0.98), August (1.00), September (1.06), October (0.93), December (0.98), January (1.38). IO ratio of VBCs at School 2 was

Table 4 Temperature and relative humidity (Rh) in different months in three schools

Months	Temperature (°C)						Relative humidity (%)					
	S*1		S2		S3		S1		S2		S3	
	I**	O***	I	O	I	O	I	O	I	O	I	O
July	32.4	35.7	32.5	36.7	31.2	36.1	78	80	78	79	78	79
August	30.2	32.5	30.7	33.2	30.5	33.8	80	83	80	81	80	81
September	28.4	30.4	28.6	31.0	28.5	30.6	71	66	71	66	71	66
October	26.3	28.6	27.0	28.1	27.2	29.0	65	58	65	58	65	58
November	20.1	22.8	20.8	22.2	23.1	24.1	50	40	50	40	50	40
December	18.5	16.2	18.5	17.2	18.7	16.2	63	67	63	67	63	67
January	21.6	20.0	21.5	20.1	21.1	20.7	60	59	60	59	60	59
February	22.4	21.0	21.0	21.9	21.4	22.8	56	60	56	60	56	60
March	24.1	28.0	24.0	28.5	24.3	28.8	46	50	46	50	46	50
April	32.8	35.0	32.1	35.3	32.9	35.9	37	35	37	35	37	35

*S = school; **I = Indoor; ***O = Outdoor

Table 5 Descriptive statistics of IO ratio of viable counts of Bacteria and Fungi

Months	Bacteria			Fungi		
	S1	S2	S3	S*1	S2	S3
N	40	40	40	80	20	20
AM*	0.98	0.89	0.98	1.16	0.98	0.91
SD**	0.30	0.195	0.306	0.301	0.259	0.218
Range	0.44-1.58	0.61-1.62	0.38-1.98	0.64-1.78	0.61-1.47	0.56-1.51
GM***	0.9333	0.8794	0.9343	1.124	0.9515	0.8872
[1]*P* value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Significant (alpha = 0.05)?	Yes	Yes	Yes	Yes	Yes	Yes
[2]*P* value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Significant (alpha = 0.05)?	Yes	Yes	Yes	Yes	Yes	Yes

*AM: Arithmetic mean; **SD: Standard deviation; ***GM: Geometric mean
[1]One sample *t*' *test*; [2]Wilcox on signed rank test

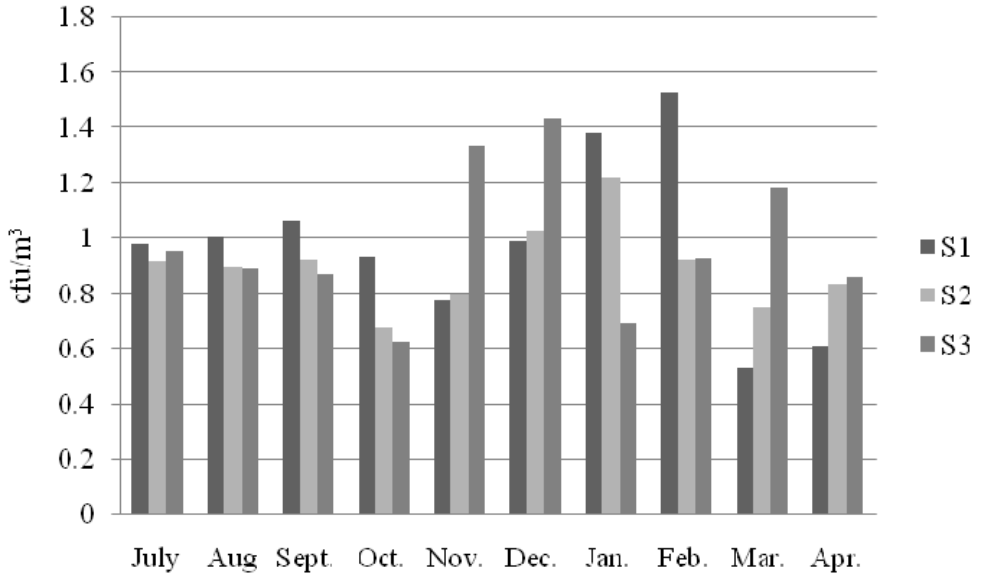

Fig. 7 Indoor to outdoor ratio (IO ratio) measured for bacteria in three schools

established in range of 0.6 to 1.2. The mean IO ratio at this place highest in month of January (1.2), followed by December (1.03), July (0.91), September (0.92), February (0.92). Range of IO ratio for VBCs at School 3 was found between 0.38-1.97. Mean IO ratio for VBCs in each month at this place found to be utmost in month of December (1.4) followed by November (1.3), March (1.18). Lowest IO ratio for VBCs at this place was calculated in the month of October (0.62), followed by January (0.69).

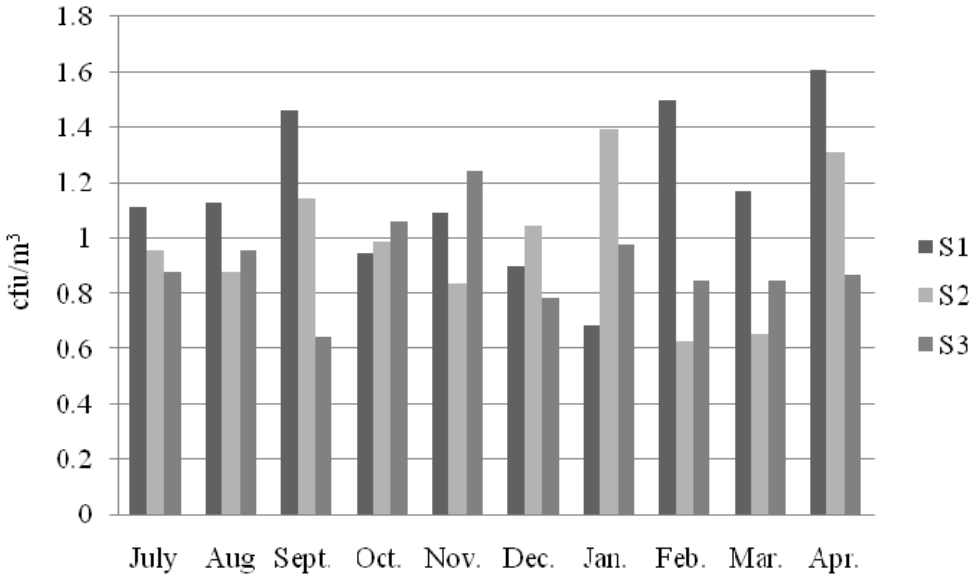

Fig. 8 Indoor to outdoor ratio (IO ratio) measured for fungi in three schools

Table 6 Spearman rank correlation coefficients for temperature (°c), relative humidity (Rh%) and concentration of viable counts of bacteria and fungi (cfu/m^3) measured inside and outside of the schools

S1	Rh (I)	Temp. (I)	Rh (O)	Temp. (O)	Bacteria (I)	Fungi (I)	Bacteria (O)	Fungi (O)
Rh (I)								
Temp. (I)	0.237^{ns}							
Rh (O)	0.879**	0.018^{ns}						
Temp. (O)	0.122^{ns}	0.939**	-.037^{ns}					
Bacteria (I)	0.742*	0.671*	0.602^{ns}	0.508^{ns}				
Fungi (I)	0.430^{ns}	0.900**	0.273^{ns}	0.902**	0.742*			
Bacteria (O)	0.624^{ns}	0.784**	0.418^{ns}	0.744*	0.802**	0.903**		
Fungi (O)	0.709*	0.705*	0.442^{ns}	0.683*	0.644*	0.794**	0.794**	
S2								
Rh (I)								
Temp. (I)	0.237^{ns}							
Rh (O)	0.879**	0.018^{ns}						
Temp. (O)	0.122^{ns}	0.939**	-.037^{ns}					
Bacteria (I)	0.709*	0.638*	0.430^{ns}	0.555^{ns}				
Fungi (I)	0.479^{ns}	0.888**	0.261^{ns}	0.872**	0.830**			
Bacteria (O)	0.612^{ns}	0.681*	0.321^{ns}	0.598^{ns}	0.939**	0.842**		
Fungi (O)	0.552^{ns}	0.736*	0.285^{ns}	0.604^{ns}	0.673*	0.794**	0.745*	
S3								
Rh (I)								

Table 6 Continued

Temp. (I)	0.237 [ns]						
Rh (O)	0.879[**]	0.018 [ns]					
Temp. (O)	0.122 [ns]	0.939[**]	-.037 [ns]				
Bacteria (I)	0.426 [ns]	0.780[**]	0.267 [ns]	0.777[**]			
Fungi (I)	0.491 [ns]	0.748[*]	0.212 [ns]	0.659[*]	0.888[**]		
Bacteria (O)	0.620 [ns]	0.716[*]	0.359 [ns]	0.651[*]	0.884[**]	0.900[**]	
Fungi (O)	0.515 [ns]	0.900[**]	0.309 [ns]	0.829[**]	0.936[**]	0.867[**]	0.912[**]

*Correlation is significant at the 0.05 level; **Correlation is significant at the 0.01 level
[1]S = School; [2]Rh = Relative humidity; [3]Temp. = Temperature; [4]I = Indoor, [5]O = Outdoor
[ns]Not Significant

IO ratio for VFCs in three schools was ranged from 0.56-1.79. Mean IO ratio for fungi in these places was calculated > 1 on thirteen times and five times were found near to 1 (1 to 0.9). The IO ratio for VFCs at School 1 was ranged from 0.64 to 1.79. Mean IO ratio for VFCs at this place has been calculated maximum in the month of April (1.61), followed by February (1.5), September (1.46). The analysis illustrated that IO ratio for VFCs at this place was frequently found to be > 1 or close to 1. Monthly analysis of IO ratio of VFCs in School 2 was ranged between 0.60-1.4. Highest IO ratio was calculated for the month of January (1.4), followed by April (1.3), and December (1.04). Mean IO ratio close to 1 was noticed for July (0.96), and October (0.99). Range of IO ratio for VFCs in School 3 was measured between 0.56-1.28. Mean IO ratio of VFCs for each month at this site was maximum for the month of November (1.24), followed by October (1.06) and close to one in January (0.97) and August (0.96).

According to Spearman correlation coefficient (Table 6), indoor and outdoor ratio for viable counts of bacteria and fungi were integrated over entire test period (ten months), was found to have significant correlation for VBCs (r = 0.802 P < 0.005) and VFCs (r = 0.794 P < 0.006) at School 1, VBCs (r = 0.939 P < 0.000), and VFCs (r = 0.794 P < 0.006) at School 2, VBCs (r = 0.884 P = 0.001) and VFCs (r = 0.867 P = 0.001) at School 3.

4. Discussion

The mean monthly concentration of TVMCs in three schools was ranged between 801.6-5368.3 cfu/m^3. TVMCs were outcome of viable counts of bacteria plus viable counts of fungi measured simultaneously at same place and same time. VBCs were constituted 63.7% of the mean total viable microbial counts where as VFCs was formed 36.3% of the total. Viable microbial concentrations reported in this study were comparable to those in other reports such as range of viable microbial counts between 100-10^4 cfu/m^3 (Bouillard *et al*. 2005, Jo and Seo 2005, Kaloogerakis *et al*. 2005).

Number of viable counts of bacteria was exceeded over the number of viable counts of fungi in this study. Similar results were reported by Gorney and Dutkiewicz (2002), Razek *et al*. [(48)]. However, the values of viable counts of bacteria and fungi in present study were not measured as higher as reported in other studies, for example Anna *et al*. (2004) reported maxima of approximately total microbial counts > 12900 cfu/m^3, containing maxima of viable bacterial

community in Poland. Kate *et al*. (2002) reported maxima of viable fungal counts were 62000000 cfu/m^3 and approximately 100000 colony forming units of viable bacteria in per cubic meter of air.

One or closer to one IO ratio indicates equilibrium between indoor and outdoor air is almost reached. Sometimes higher indoor concentration of viable counts of bacteria and fungi than outdoor air indicate an evidence of presence of source contributing indoor viable microbial community, as IO ratio is 1 or quite close to 1 (Figs. 7 and 8).

Microbial flora of indoor air depend on several factors including the number, hygienic standard of people present, the quality of occupational system and mechanical movement within the enclosed space. Table 1 illustrates the indoor conditions, cleaning methods and activities observed during the study inside three schools. Human activities like talking, walking, laughing, frequent movement of children in classrooms, sweeping and dusting were brought re-dispersion and re-aerosolization of dust and enhancing the source strength. These activities lead to IO ratio greater than 1. This assertion was supported by previous investigations like presence of people is the most significant parameter resulting in elevated with bioaerosol counts in the absence of significant indoor or outdoor sources (Jo and Seo 2005, Kalogeraki *et al*. 2005, Zilma *et al*. 2005).

Moreover respiratory tract derived bacteria of children and presence of food particles left behind in classrooms by the children were recognized as source and sink of microbes to be breed over there. Besides, dampness is an indicator of poor ventilation, resultant increased levels of a wide range of potentially harmful airborne viable micro-biota (Anna *et al*. 2004, Lignell *et al*. 2007, Meklin *et al*. 2002, Park *et al*. 2006) similar to the observations noticed in this study for Schools 1. Furnishings and textiles in the classrooms also act as significant reservoirs of bio-contaminants (Smedge and Norback 2001).

The higher mean concentration of viable bacteria and fungi per cubic meter of air in indoors during the winter was shown the consistency to the results obtained in the other occupational environment (Nielsen 2009, Pantelic 2009). It might be bias of air exchange from outdoor to indoor and vice-versa due to not enough ventilation, closing of doors and windows during winter.

Temporal factors like monthly temperature and Rh (Table 4) were appeared to have influence on concentration of VBCs and VFCs. Outdoor and indoor air temperature significantly correlated (Table 6) with the concentration of culturable airborne fungal flora isolated from three schools ($p < 0.01$, $p < 0.05$). The outdoor viable counts of fungi in School 1 and School 3 was shown a strong positive correlation for outdoor temperature ($p < 0.05$, and $p < 0.01$). It was noted that temperature had correlated better with the concentration of airborne fungi and bacteria than relative humidity. This finding was confirmed with the findings of Adhikari *et al*. (2005), Taekhee *et al*. (2006). However, in a study Tsai and Liu (2009) were found that relative humidity was exaggerated fungi levels more than temperature did. The indoor viable bacterial counts showed a significant correlation with the indoor temperature ($p < 0.05$, $p < 0.01$). Hong Zhu *et al*. (2003) were found direct to have influence of environmental factors on the bacterial concentration in indoor environment. Airborne bacterial counts were directly associated to the temperature and relative humidity (Matkovic *et al*. 2006). The outdoor Rh had pronounced effect on the concentration of outdoor bacteria (Hong Zhu *et al*. 2003). As regard to monthly variations, it was evident that number of viable bacterial and fungal counts may not be reflection of the relative humidity of the environment.

Total viable counts of bacteria in a moldy problem homes were relatively on the higher range as compare to non-moldy dwellings (Gorny and Dutkiewicz 2002). These findings could be explained that higher range of fungal counts was an important impact in the development of indoor bacterial concentration, too. In the study, indoor fungal concentrations was shown a positive

correlation with indoor viable counts of bacteria ($p < 0.05$, $p < 0.01$).

Indoor concentration of viable microbial counts in all schools were significantly associated with the outdoor level of fungal counts ($p < 0.01$). It appears that indoor level of culturable counts was also dependent on outdoor concentration. The study got support with the previous studies that distribution of indoor air borne microbes was depended on outdoor air (Bonetta *et al*. 2009, Mota *et al*. 2008, Ping Ren *et al*. 1999).

The pollution of occupational environment by fungi and bacteria is an important factor affecting health as a result; diseases such as allergy, rhinitis, bronchial asthma, respiratory tract infection may develop in occupants (Curtis *et al*. 2004). Types of fungal species isolated and identified in the three schools, were already characterized as potential allergenic in nature and exposure to them may provide immune responses in the susceptible individuals, were found to be capable of eliciting a number of diseases responses such as infectious, allergic and toxic effects (Albinas *et al*. 2004, Hamilos 2010, Northcut *et al*. 2004, Madani *et al*. 2010, Rogers 2003). Higher concentration of biocontaminants unequivocally associated with exacerbations of asthma and respiratory problems among children (Kim *et al*. 2007, Pongracic *et al*. 2010). Prolonged exposure to variety of bacteria and fungi were produced chronic health problems among children (Barnes 2008, Inal *et al*. 2008). Exposure to higher concentration of several microbial community and their toxins may result in adverse health effects and may pose a potential risk of several diseases for the occupational environment (Anna *et al*. 2004, Kim *et al*. 2007, Zofia *et al*. 2003).

Children of school age are still developing physically, and may be more likely than adults to suffer adverse effects as a result of poor IAQ (Daisey *et al*. 2003, Mendell and Heath 2005). Also poor air quality in schools can affect children's desire and ability to concentrate, learn and may lead to increased rate of absenteeism. Children are more susceptible to microbial exposure in school environment (Kim *et al*. 2007, Zhao *et al*. 2008).

While indoor environment is considered to be protective, presence of microbial community at high concentration may confer more serious risks than those related to outdoor exposures, whenever their concentrations exceed to recommend maximum limits set by various standards. According to a report of American Conference of Government Industrial Hygienists (ACGIH) (1989) the indoor airborne microbial level is normally less than one-third of the outdoor level. If the IO ratio exceeded this value than the remedial action should be taken to identify the source of emission and methods to reduce the counts should be instigated. Schools examined in this study have been required to give attention regarding IAQ analysis and exposure study.

European commission Report (CEC 1994) had decided following limits for bioaerosol; 0 undetectable, 1-499 cfu/m^3 low, 500-999 cfu/m^3 medium and > 1000 cfu/m^3 high. National Institute of Occupational Safety Health (NIOSH) has been set 1000 cfu/m^3 for total number of bioaerosol particles. American Conference of Governmental Industrial Hygienists (ACGIH) with the culturable counts for total bacteria not to exceed 500 cfu/m^3 (Jensen and Schafer 1998). WHO (1990) has set the guidelines of bioaerosol counts at 500 cfu/m^3. In the study, indoor level of viable counts of bacteria and fungi during the surveyed period sporadically were exceeded the concentration level > 1000 cfu/m^3.

4. Conclusions

It is advisable that strict measures should be put in place to check the increasing microbial load in the school environment. This is necessary, because school is a place where children go to

promote their life, may serves as an avenue to contact diseases and diminish the children health.

Due to higher concentration of viable counts of bacteria and fungi and lack of standard guidelines of those indoor contaminants in schools with respect to Indian conditions, it is strongly recommended that comprehensive exposure assessment program in schools is required in order to determine whether exposure of indoor air is able to cause risks in children; to set up standards/guidelines and permissible limits for the concentration of microbial community for schools environment. Since, assessment of airborne dust carrying both culturable and non-culturable microbial communities' serve as a better indicator for managing and abating the contemporary health risks and also quality of indoor air.

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