

## Original Research Article

### Microbial air contamination in a school

Kavita Naruka\* and Jyoti Gaur

Department of Zoology, Jai Narain Vyas University, Jodhpur, Rajasthan, India

\*Corresponding author

#### A B S T R A C T

##### Keywords

Airborne;  
Contamination;  
Indoor air;  
*Staphylococci*  
and  
*Micrococcii*.

The presence of bacteria and fungi in indoor air pose a serious problem from the point of view of health protection and environmental engineering. Precise determination of various groups of microorganisms indoors is necessary for both to estimate the health hazard and to create standards for indoor air quality control. This is especially important in such densely populated facilities like educational institutions. In present study, the level of microbial contamination in the air of a senior secondary school in Jodhpur, Rajasthan (India) was estimated. The overall counts of Gram positive organisms were found to be higher than Gram negative organisms. Of these, *Staphylococci* and *Micrococcii* were predominant Gram positive bacteria, while *Pseudomonas* sp. and *Enterobacter* sp. were predominant potentially pathogenic Gram negative bacteria isolated from the air samples. Among fungus *Aspergillus* sp. was found to be the most common.

#### Introduction

Indoor air quality has become an important public health concern as most people spend more than 90% of their time in indoors like houses, offices and schools. Air in the indoor environment can be polluted by a number of pollutants among which airborne microorganism (bacteria and fungi) are one of the most important. It has been estimated that one-third of indoor air quality (IAQ) complaints may be due to microbial contamination (Pope *et al.*, 1993) and exposure to these may cause allergies, respiratory and immunotoxic diseases (Douwes *et al.*, 2003). Several investigators have reported these contaminants in different indoor environments such as hospitals

(Gunderman, 1974), schools (Bartlett *et al.*, 2004) and offices (Morey *et al.*, 1986).

Schools are public places inhabited by thousands of students every day and tend to have high levels of activity that typically result in higher levels of airborne fungi and bacteria. The amount of the microbial content of indoor air of school is an important parameter because it has a direct impact on the mental health, physical development and performance of the students.

Numerous studies have shown that occurrence of moulds in the air of school poses a great risk to children. Childhood

asthma is one of the commonest chronic respiratory disorders in Asian countries (Kusunoki *et al.*, 2009; Amarasekera *et al.*, 2010; Tsai *et al.*, 2010) and its prevalence and severity have been reported as increasing in many countries (Quah *et al.*, 2005; Lai *et al.*, 2009; Koshy *et al.*, 2010). Richards (1986) also noted that allergic disease (nasal allergy, asthma, and other allergies) is the “number one” chronic childhood illness, accounting for one-third of all chronic conditions occurring annually and affecting 20% of school children. Taskinen *et al.* (2000) reported positive reaction to fungal allergens in skin prick tests and serum IgE reactions in 14% of school children. Platt *et al.* (1989) revealed that elevated occurrences of wheezing and fever in children was connected with high numbers of fungi in the air. The objective of the present study is quantitative and qualitative determination of airborne microorganisms at a school classroom and to study their variability in different seasons.

## Materials and Methods

Samples were taken in a school room, monthly from July 2010 to June 2011, by natural sedimentation method. Nutrient agar (NA) (HiMedia Laboratories Limited, Mumbai, India) was used for the sampling and cultivation of bacteria. For isolation of fungi, Potato dextrose agar (PDA) (HiMedia) supplemented with 10 mg/L chloramphenicol was used. Duplicate Plates of NA and PDA were exposed to air for 30 min and were setup at a height representative of the normal human breathing zone, that is, 1.5 m above floor level. Nutrient agar plates were incubated at 37°C for 48 h to allow the growth of aerobic bacteria, while PDA plates were incubated for up to 5 days at 25°C to allow

the growth of fungal colonies. The average of colony forming units (CFU) of both bacteria and fungi was calculated and converted to organisms per cubic meter of air (CFU/m<sup>3</sup>).

Bacterial colonies were initially characterized by morphology and microscopic appearance, and identified further by biochemical tests according to Bergey's Manual of Systematic Bacteriology (Sneath *et al.*, 1986). A wet mount preparation of each fungal colony was prepared by using Lacto phenol-cotton-blue solution and examined microscopically. Identification of fungi was based mainly on growth colonial appearance, microscopic examination of the spore and hyphal characteristics of the stained preparations (Frey *et al.*, 1979). During the whole study, parameter such as temperature and humidity was also measured monthly. These parameters were then statistically correlated with observed data of study.

## Result and Discussion

The fluctuations in the monthly concentrations of bacteria in the air of the school are presented in Figure 1. The concentrations of airborne bacteria ranged from 198.33–347.5 cfu/m<sup>3</sup>, with peak values in September and the lowest values in April. The most common bacteria isolated were Gram positive cocci which accounted for 47.08% (Fig. 2) of the total airborne bacterial count. Prevailing isolates were the *Staphylococcus aureus*, while the remaining part of this group belonged to *Staphylococcus epidermidis*, *Micrococcus luteus* and *Micrococcus kristinae*. Gram negative bacteria constituted 28.98% (Fig. 2) of the total bacterial count. *Pseudomonas sp.* was commonly isolated followed by

*Enterobacter aerogenes*. The other isolates were identified as *Escherichia coli* and *Serratia marcescens*. Endospore forming Gram positive bacilli (*Bacillus* sp.) were the third group which constituted 23.94% (Fig. 2) of the total bacterial count. They comprised the species *Bacillus subtilis*, and *Bacillus megaterium* and *Bacillus licheniformis*.

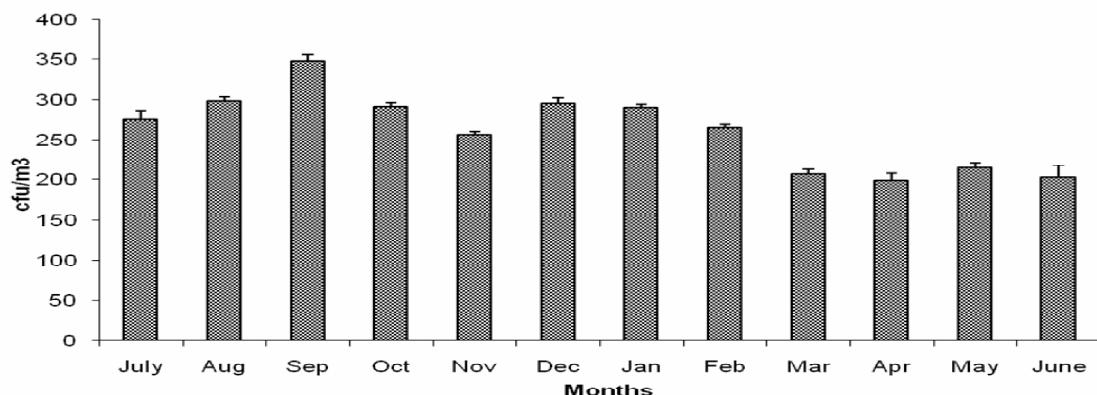
The fluctuations in the monthly concentrations of airborne fungi in the air of the school are presented in Figure 3. The concentration of airborne fungi ranged from 35.83–81.66 cfu/m<sup>3</sup> with the very distinct peak in December and the lowest value in April. Among the fungus the prevailing species was *Aspergillus* which formed 45.35% of total fungal count and detected throughout the year, comprised *A.niger* , *A.fumigatus* ,*A.terreus* and *A.flavus*. Other fungal isolates were *Penicillium* sp., *Rhizopus* sp., *Alternaria* sp., *Helminthosporium* sp., *Cladosporium* sp. and *Fusarium* sp.

The seasonal variability in conjunction with the types of airborne bacteria and fungus was also recorded. During summer and monsoon the concentration of airborne Gram positive cocci and Gram positive bacilli were dominant whereas during winter, Gram negative rods were also increased significantly. In fungus *Aspergillus* count dropped in winter whereas other fungal species were higher in this season (fig.4). Correlation of temperature was negatively strong with total airborne bacteria (- 0.42601) and with Gram negative bacteria (-0.80932) but positively weak with Gram positive bacteria (0.02918).Humidity was correlated positively strong with total airborne bacteria (0.78239), Gram negative (0.65312) and Gram positive (0.64972) bacteria. On other hand

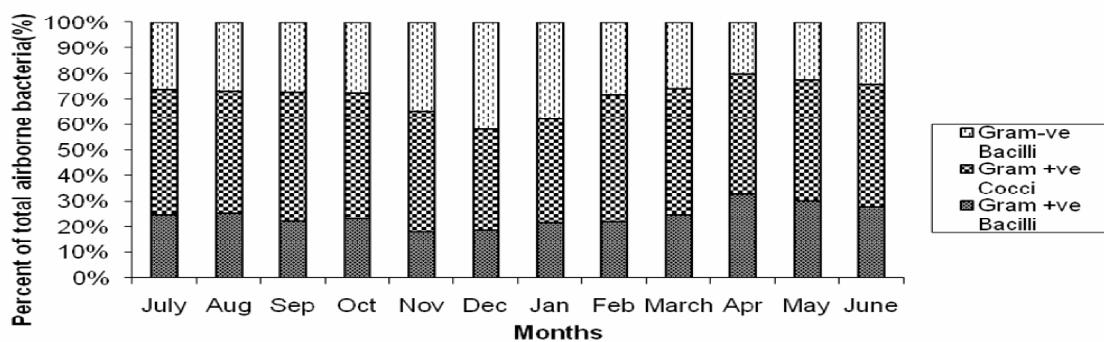
Correlation of temperature was negatively strong with total airborne fungal count (-0.75697) and with other fungal species count (-0.77574) but positively strong with total *Aspergillus* count (0.75129) whereas, humidity was correlated positively weak with both total fungal count (0.29729), and other fungal species (0.24539), but negatively weak with total *Aspergillus* count (-0.13629).

Microbiological indoor air quality of a school is an important factor for children's health, as school serve a daily environment for them. Microbial concentration of indoor air of the school is affected by many factors, including human activity, the age of school building, ventilation conditions, outdoor air, season (primarily temperature and humidity), etc. (Thorstensen *et al.*,1990). In the present study the prevalent bacteria isolated were Gram positive cocci belonging to saprophytic micro flora generally associated to human skin and mucosa which can be dispersed through droplets or skin peeling and maintained in air. Stryjakowska-Sekulska *et al.* (2007) observed a significant increased concentration of bacteria and fungus in afternoon (during the lessons) as compared to morning in various rooms of a university. Soto *et al.* (2009) found the same result, concluding that bacterial contamination into indoor air derives from human presence. The association of airborne microbes and human activity has also been reported by many studies. Karwowska (2003) reported highest level of bacteriological contamination in corridor and rooms during lessons in a school.

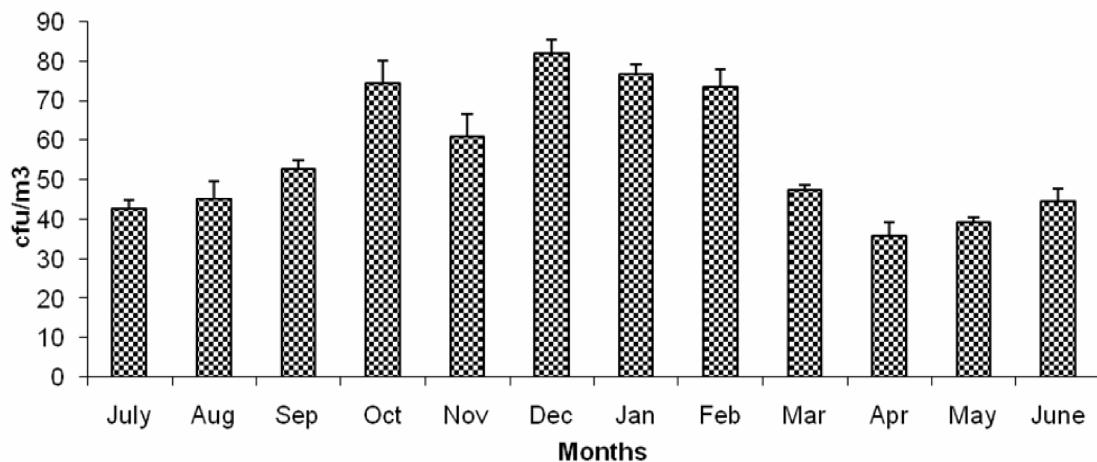
**Fig.1** Monthly fluctuation in concentration of total airborne bacteria in the school (mean  $\pm$  S.D.)

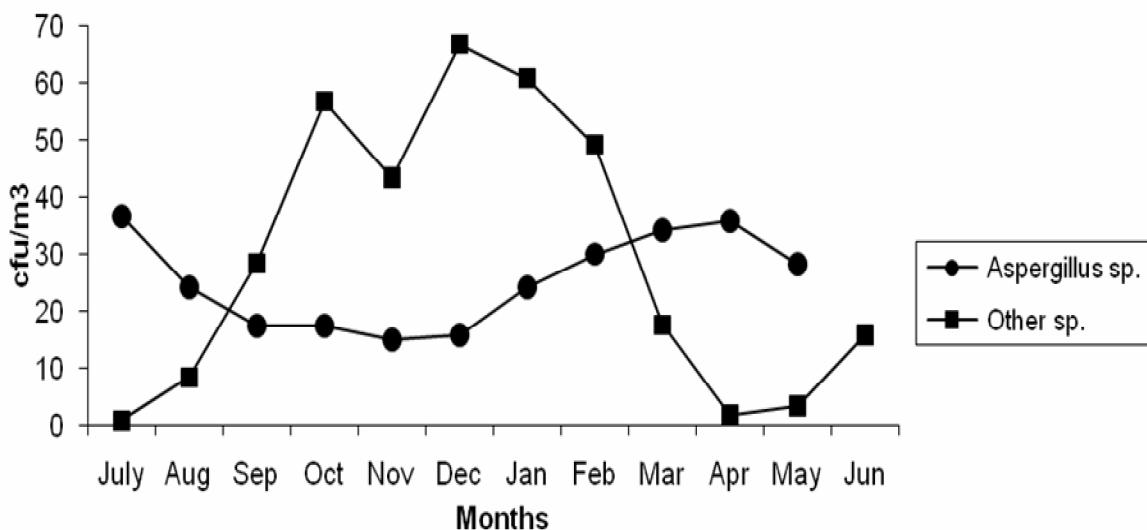


**Fig.2** Distribution of various groups of airborne bacteria in each month



**Fig.3** Monthly fluctuation in concentration of total airborne fungi in the school (mean  $\pm$  S.D.)



**Fig 4** Monthly concentration of *Aspergillus* sp. versus other sp. in the air of school.

Viability of bacteria has shown too affected by environmental temperature. Webb (1959) observed that the prevalence of airborne bacteria was immediately related with climatic conditions primarily temperature and humidity. The survivability pattern of airborne bacteria in present study shows that temperature and humidity had pronounced effect on survival of most of these airborne bacteria. Since the group of Gram positive cocci and other Gram positive bacteria which have mechanism to resist the desiccation factors they were prevalent in summer and monsoon season. Gram negative bacteria and members of the group enteric bacteria could only survive in low temperatures and moderate humidity which is a major factor for dissemination and distribution of Gram negative bacilli, especially members of the enterobacteriaceae in winters (Pathak and Verma, 2009).

Results of this research showed that most common fungus in schools are from genera *Aspergillus* and *Cladosporium* sp. followed by *Fusarium* sp. Dotterud *et al.* (1995) also noticed that most

commonly occurred moulds in Norwich schools belonging to the genera: *Penicillium*, *Aspergillus*, *Cladosporium* and *Mucor*. Fungal flora of the air of examined school room was dominated by *Aspergillus* sp. and isolated throughout the year. The present results are in agreement with those of Augustowska and Dutkiewicz, (2006) who found the same results in other indoor setting.

According to some previous studies the microbiological quality of indoor air is highly influenced by the microbiological composition of outdoor air, which itself very much influenced by environment, season, the weather and even daytime. In present study *Cladosporium* sp. and *Alternaria* sp. so-called "outdoor moulds" occurred from September to February (Rainy and winter season), comprises the academic period in which density of people was very high. This may be due to continuous input of microorganisms from outside via visiting people (Stryjakowska-Sekulska *et al.*, 2007)). This result provided the evidence that high concentration of fungi in atmosphere can

influence microbiological indoor air contamination.

As there are no generally accepted threshold limit values concerning concentrations of bacteria and fungi in the air of indoor, the obtained results could be compared only with the values recommended by various authors or institutions. According to indoor air quality standards suggested by Environmental Protection Administration (EPA) Taiwan, indoor air of schools and other educational settings should have less than 500 cfu/m<sup>3</sup> of total bacteria count and 1000 cfu/m<sup>3</sup> of total fungi count ([taqm.epa.gov.tw/taqm/en/Default.asp](http://taqm.epa.gov.tw/taqm/en/Default.asp)). In Hong Kong and Singapore good microbiological class air should include less than 1000 cfu/m<sup>3</sup> and 500 cfu/m<sup>3</sup> of bacteria respectively ([iaq.gov.hk/tables.html](http://iaq.gov.hk/tables.html); Obbard and Fang, 2003). According to the guideline of American Conference of Governmental Industrial Hygienists (ACGIH) fungal spores in residential buildings should be less than 500 cfu/m<sup>3</sup> and for commercial buildings the limit is 250 cfu/m<sup>3</sup> (ACGIH, 1999). In our research, however the levels of bacteria and fungus, mentioned above were not exceeded but, showed considerable number in the air of school. According to some molecular findings the level of total bacteria may be even up to 5-times higher than the number of culturable bacteria determined as CFU/m<sup>3</sup> (Amann *et al.*, 1995), so the present results may be an under estimate of real indoor air microbial concentration. Therefore, it is necessary to control microbial air pollution in such educational settings and also to develop the standards related to it.

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