

Research Article

Indoor Air Quality in Naturally Ventilated and Mixed Model Ventilation in Public University of Pakistan

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Airborne microorganisms have potential to cause infections and respiratory diseases like asthma. It is necessary to reduce the human exposure to such pathogens. This study was based on the determination of indoor environmental quality and the quantification of microbial load in a university premises in Karachi, Pakistan. Seven different locations with high human occupancy during office hours were sampled in winter and summer. Culturable microorganisms i.e. bacteria and fungi were collected on selective media by Spin Air V2 sampler. The average bacteria and fungi concentration in winter were 133 ± 26 colony forming unit per cubic meter (CFU.m⁻³) and 199 ± 59 CFU.m⁻³ respectively. In summer counts were higher than winter with bacterial and fungal count of 205 ± 39 and 306 ± 102 CFU.m⁻³. There was strong correlation ($r = 0.8$, $p = 0.032$) between fungal count and humidity, and between bacterial and fungal count ($r = 0.802$, $p = 0.030$). The most common culturable airborne fungi in both seasons were *Aspergillus*, *Penicillium*, *Cladosporium*, *Fusarium*, *Alternaria*, *Stachybotrys*, *Candida*, and *Rhodotulora*. Results indicate that the indoor environment in university premises harbours high concentrations of microorganism and needs regular indoor air quality monitoring and establish local guidelines to prevent diseases.

Keywords: Airborne; Indoor environmental quality; Microbial load; Bacteria; Fungi; Humidity**Introduction**

Microorganisms are inevitable in enclosed environment and air due to their presence in nature [1-3]. Indoor work places are continuously challenged by microorganisms including bacteria and fungi which can deteriorate the indoor environmental quality. *Alternaria*, *Aspergillus*, *Penicillium* and *Cladosporium* are common filamentous fungi (Mold) found in indoor environment [4]. They are known as allergen fungi and isolated from patients having chronic sinusitis [5]. The standard for indoor environment stated that pathogenic and toxigenic fungi such as *Aspergillus fumigatus* and *Stachybotrys atra* are not permitted in indoor environment [6]. According to WHO [6] if single species present at a concentration of > 50 CFU.m⁻³ investigation should be carried out properly. In the case of multiple species, 150 CFU.m⁻³ is acceptable, while if only common phylloplane fungi are present in indoor environment such as *Cladosporium* then 500 CFU.m⁻³ is acceptable.

Common bacteria of indoor environment are members of Propionibacterineae, Xanthomonadaceae, Micrococccineae, Enterobacteriaceae and Corynebacterineae [7]. *Sphingomonas*, *Caenibacterium*, *Staphylococcus* [7] and *Streptococcus* species are also found [8]. Gram-positive bacteria found in indoor environment are members of Phylum Firmicutes, while Gram-negative includes the family Oxalobacteraceae, Comamonadaceae, Neisseriaceae and Rhizobiaceae [8].

It is evident from previous studies that presence of toxigenic fungi [9-11] and bacteria [12] is significantly influenced with environmental factors such as dampness and humidity. Dampness,

ventilation, heating and cooling systems [13] can cause Sick Building Syndrome (SBS), organic dust toxic syndrome and respiratory infections i.e. Legionellosis, humidifier fever, and hypersensitivity pneumonitis [14]. These infections can be transmitted through the air, affecting people working in this environment or associated with the building [15].

Dampness is measured as relative humidity in indoor environment. The USA Environmental Protection Agency allows 30-50% relative humidity [16] while the International Energy Agency allows up to 80% [17]. An association has been reported not only between dampness and mold growth in indoor environment [4], but also between dampness, mold growth and respiratory symptoms [18,19]. Relationship was found between dampness in buildings and asthma [20] and sinusitis [21]. Prolonged exposure with mold in damp building can result in bronchial obstruction [22-23]. Indoor humidity and mold are associated with increased exacerbation, dyspnea, wheeze, cough, allergic rhinitis, eczema, and upper respiratory tract symptoms [12].

Temperature in indoor environment occurs in wide range which is favourable for the growth of microorganisms specially fungi. Most indoor fungi grow best between 10-35°C and cause considerable health effect such as hypersensitivity pneumonitis [13]. Human occupancy causes an increase in bacterial load [15]. Another important factor which determines the indoor microbial communities is ventilation which increases air borne bacteria even when there is no human occupant [24]. Good ventilation in indoor environment in university buildings has positive impacts on human health and productivity [24]. Improper ventilation can cause tiredness, headache, immunity

weakness and sick building syndrome. It can affect the building structure and associate with mold growth [25]. Temperature, wind speed and air pressure have impacts on ventilation in indoor environment [25]. Presence of fungi in indoor environment is found to be positively associated with temperature and air exchange rate [26]. Increased wind speed resulted in decrease CO₂ concentrations in indoor environment. Well design and well maintained buildings are necessary for the prevention of microbial growth (WHO, 2009).

Seasons with high microbial concentration can cause more infections [26]. Concentration and type of fungi were found to be higher in winter [27]. *Cladosporium* was dominant in summer in indoor environment while in winter *Penicillium* and *Aspergillus* were common [27]. Study of indoor environment of homes indicated significant seasonal variation in fungal count; high in summer and low in winter, while indoor bacteria were highest in spring and lowest in summer [26]. Increase in fungal spore count due to season can result in increased asthma in children [28]. Firmicutes were dominant in winter while proteobacteria were more common in summer [8].

Three different ventilation systems viz. natural ventilation, mechanical ventilation and mixed model ventilation are normally utilised in commercial and non-residential building [29]. In natural ventilation, windows, doors, skylights and roof ventilators supplies an ample amount of air for building. In mechanical ventilation, air is supplied, conditioned and thermally regulated with Heating and Ventilating Air Conditioning system (HVAC). The HVAC system intake outdoor air followed by filtration, heats/cools, dehumidifies/humidifies and distribute through duct network to air vents located in the building. In mixed model ventilation very common in Pakistan, combining natural and mechanical ventilation system through use of window unit type air conditioners in high use spaces but with a substantial and highly variable natural ventilation component through opening windows and doors. Presence of bacteria and fungi in the indoor environments such as schools, offices and residences increases the chances of exposure to harmful bio-aerosols and thus becomes a public health concern [30]. There is a need for understanding of the likely association between indoor air pollutants, environmental factors and human health, regular monitoring and quantification of exposure to airborne microorganisms.

In Pakistan, especially in highly populated city like Karachi, indoor air quality is a growing concern and published literature regarding indoor air pollution is limited [31]. Karachi has a warm humid climate with short spell of winter (December to February). Karachi air quality is relatively low compared to the rest of the World. The main contributing source of Karachi's air pollution is fossil fuel combustion, specifically vehicle and industrial exhaust. Also, post-harvest open burning of agricultural fields in winter can cause severe pollution events for a few days in a year. The ambient indoor air quality across Karachi has not been studied in the literature and the contribution of outdoor air pollutants to indoor environment had not been described by previous studies. The aim of this bio-aerosol sampling was the quantitative evaluation of the viable airborne bacteria and fungi in university premises in winter and summer seasons in Karachi and to determine the relationship between concentration of microorganism and different environmental factors. The hypothesis of this study was that the environmental factors such as humidity and temperature are the major reasons for the increased microbial count

in indoor environment in university premises. Besides the standard enumeration of culturable microbes as CFU.m⁻³, this study attempted to analyse various environmental factors including temperature, relative humidity, dew point, barometric pressure and wind-speed to find the relationship.

Materials and Methods

Sampling Location

The bio-aerosols samples were collected from public sector university premises having more than 10,000 students and staff. Most of the students and staff come from all over the city. Seven different categories of indoor areas with mix and mixed model ventilation system were selected for airborne microorganisms and microclimatic parameters monitoring. These include class rooms, laboratories, offices, canteens, common rooms, libraries and computer labs. Twenty-one different locations (n=3 for each category) were sampled in duplicate on two different days in NED University of Engineering and Technology, Karachi in winter and summer seasons during months of December and January for winter sampling and April and May 2019 for summer. Attributes of the sampled locations are given in Table 1. In order to maintain the uniformity, all samples for microclimatic and bio-aerosol analysis were collected on the same day. That is, a total of 21 samples (3 from each category) were collected on the same day in summer and then it was repeated in winter.

All bio-aerosol samples were collected in working hours using the Spin Air V2 (IUL, USA) sampler to assess the indoor airborne concentration of cultivable bacteria and fungi. The sampling height which was approximated to human breathing zone and at the center of the room. For bio-aerosol analysis, 300 litres of air were sampled using the Spin Air V2 sampler at a rate of 60 L.min⁻¹ on selected agar medium on the culture plate [32]. For uniform air impact on sampled agar media, the plate was rotated at 2 rpm. Before or after each sampling, the sampler surface was disinfected with a 70% ethanol solution to avoid the contamination.

The microclimatic parameters; wind speed, temperature, relative humidity, dew point and ambient pressure were measured using the 4000NV, Kestrel, Pocket Weather Tracker. All samples were collected during working hours or when human occupancy was involved (8:30 am – 2:30 pm). Samples were collected in duplicate in centre of the room well above the ground level (approximated to human breathing zone).

Enumeration of Bacteria and Fungi

Total culturable bacteria and fungi were impacted onto Tryptone soya agar (TSA, Merck) [33] and Malt extract agar (MEA, Merck) [34], respectively. TSA plates were incubated at 37°C for 24 h and MEA plates were incubated at 25°C for 72 h for the development of individual colonies. Colonies were counted by colony counter and colony forming units per cubic meter of air (CFU. m⁻³) were calculated using the volume of air sampled for both bacteria and fungi. Relative microbial abundances were calculated by the formula (number of colonies of a genus x 100/ number of colonies of all genera [35]. Microscopically, fungal genera were identified to genus level based on growth characteristics, colony morphology, and pigmentation on agar media and examination of colonies by lectophenol cotton blue for microscopic morphology and spore pattern. Published material

was used for the identification of fungal genera [36-37].

Statistical Analysis

Statistical analysis was carried out by using Minitab 16. Pearson's correlation ($\alpha=0.05$) was calculated between bacterial and fungal concentrations and meteorological parameters in indoors environment to determine the relationships between two factors (bacterial concentration vs. humidity, fungal concentration vs. humidity, etc.). Two sample *t-test* ($\alpha=0.05$) was performed to determine the difference between studied parameter during winter and summer seasons. All statistical tests were based on 95% confidence interval.

Results and Discussion

Bacterial Count in Indoor Environment in Summer and Winter

Table 2 shows the bacterial counts from seven different places in winter and summer seasons in the university premises. As can be seen in the table, in the winter season, common rooms are the places with highest number of bacterial counts, followed by class rooms and teacher offices. Also, as shown in Table 2, laboratories, libraries and computer labs have similar microbial counts. It is interesting to observe that the canteens, despite having high population density, have lowest number of bacteria. Canteen owners might be following the good hygiene rules and cleaning the places regularly which resulted in low bacterial count as compare to other places in University premises. The canteens surfaces are cleaned regularly multiple times in a day as compare to library, offices and other places where surfaces are cleaned only once during a working day.

In summer, as shown in Table 2, the highest average bacterial

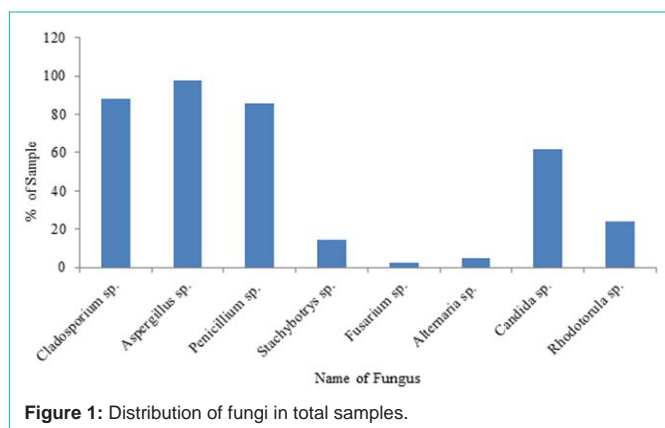


Figure 1: Distribution of fungi in total samples.

Table 1: Attributes of the sampled locations.

Categories	Sampling points	Material	Floor type	Ventilation type	Surface area (m ²)	Estimated population density (persons/10 m ²)
Class rooms	3	Brick	Tiled	Natural	67	60
Laboratories	3	Brick	Linoleum	Mixed model ventilation	89.2	35
Offices	3	Brick	Tiled	Natural	22	10
Canteens	3	Brick	Tiled	Natural	150	100
Common rooms	3	Brick	Tiled	Mixed model ventilation	110	100
Libraries	3	Brick	Tiled	Mixed model ventilation	250	100
Computer labs	3	Brick	Timber	Mixed model ventilation	150	50

count of $253 \pm 121 \text{ CFU.m}^{-3}$ was observed in faculty offices. A vast difference in microbial count was also observed among these offices, which is indicated by the relatively high Standard Deviation (SD) values. The lowest count in offices was $113 \pm 7.0 \text{ CFU.m}^{-3}$ while the highest count observed was $493 \pm 18.1 \text{ CFU.m}^{-3}$. Several environmental conditions such as ventilation, human occupancy and dampness might be the reasons for this variable count. Proper ventilation can reduce the microbial count in indoor environment. On the other hand, human occupancy [15] and dampness [4] can increase the microbial load. Table 2 also shows that the classrooms and common rooms have almost similar bacterial counts. High human occupancy might be a reason for the increase microbial count in these areas of University and both of there are cleaned once a week. Moreover, after finishing the working hours, the rooms were locked and opened again on next day.

Table 3 shows the morphological identification of bacteria in the indoor environment. As indicated in the table the Gram-negative short rods were the most dominant group in the university indoor environment and their relative abundance was 45.2%. Relative abundance of bacteria with respect to total bacteria indicates that the bacilli in chain and filamentous bacteria are also common in indoor places; 16.7 and 11.9%, respectively. Outdoor air might be the reason of their presence in indoor air. Several studies indicate that the outdoor air determines the composition of indoor air [24,38-39].

Fungal Count in Indoor Environment in Summer and Winter

Fungi growth on Malt extract agar in winter season showed that fungal count was highest in class rooms ($276 \pm 61 \text{ CFU.m}^{-3}$) followed by laboratories and offices (Table 2). Lowest numbers of fungi ($120 \pm 27 \text{ CFU.m}^{-3}$) were isolated from computer labs which have mixed model ventilation systems for cooling. Low temperature due to air conditioners might be the reason of low fungal count as same was the case in summer season when lowest count was observed in computer labs which was $162 \pm 30 \text{ CFU.m}^{-3}$ (Table 2). On the other hand, the high fungal count in summer season was found in laboratories, classrooms and offices which were 424 ± 87 , 412 ± 52 and $351 \pm 149 \text{ CFU.m}^{-3}$, respectively. This indicates that the presence of fungi was a continuous issue in these places irrespective of seasons. Contrarily to this, bacterial count was variable in sampling places in both seasons. Mean values for temperature, humidity, barometric pressure, dew point and wind speed are presented in (Table 4).

Morphological analysis indicates that among the fungal groups grown on the MEA media, 78.4% were molds (filamentous fungi) while only 21.6% were yeast colonies (Table 5). Thus the mycelial

Table 2: Bacteria and fungi on selective media during winter and summer seasons from different locations in University (n=21 sets).

Sampling points	Bacterial count on TSA (CFU.m ⁻³ ±SD)		Fungal count on MEA (CFU.m ⁻³ ±SD)	
	Winter	Summer	Winter	Summer
Class room	162±30	232±11	276±61	412±52
Laboratory	112±1	214±48	274±52	424±87
Office	151±76	253±121	208±46	351±149
Canteen	107±25	188±24	150±18	281±79
Common room	168±67	231±57	174±32	319±35
Library	114±19	143±13	193±20	189±48
Computer lab	118±26	170±44	120±27	162±30

TSA: Tryptic soya agar, MEA: Malt extract agar, CFU: Colony forming unit

Table 3: Relative abundance of bacteria in indoor environment.

Organisms	Organisms Morphology	Relative abundance
Gram positive	Bacilli in chain	16.7%
	Filamentous / branching bacteria (Actinomycetes)	11.9%
	Diplococci / tetrads	9.5%
	Cocci in bunches	7.1%
	Coccobacilli	4.8%
	Diptheroid rods	4.8%
Gram negative	Scattered short rods	45.2%

molds were the most dominant group of fungi in indoor environment in university premises. Morphological study for the identification of fungi genus showed the presence of *Aspergillus*, *Penicillium*, *Cladosporium*, *Fusarium*, *Alternaria*, *Stachybotrys*, *Candida*, and *Rhodoturolo* species. Molds were isolated from all sampling places while yeast was found in 61.90% of the sampling areas. Among molds, *Cladosporium* species were the most dominant mold and were isolated in 88.1% of samples. It was followed by *Penicillium* and *Aspergillus* species which were isolated from 83.3% and 76.2% of the samples, respectively. Isolation of *Stachybotrys*, *Alternaria* and *Fusarium* species were relatively low and they were present in 14.3%, 4.8% and 2.4% of samples, respectively. *Candida* species were present in 61.9% samples while *Rhodoturolo* species were found in 23.8% samples (Figure 1). Relative abundance of each fungus count with respect to total fungi count isolated from different sampling sites from University premises was as follow; *Cladosporium* sp.= 30.7%, *Aspergillus* sp.= 22.3%, *Penicillium* sp. = 22%, *Stachybotrys* sp.= 2.6%, *Alternaria* sp.= 0.7%, *Fusarium* sp.= 0.03%, *Candida* sp. = 21% and *Rhodoturolo* sp. = 0.6% (Table 5).

Table 4: Temperature, humidity, dew point, barometric pressure and wind speed (Mean ± SEM) at different locations in University premises.

Sampling points	Temperature (°C)		Humidity (%)		Dew point (°C)		Barometric Pressure (psi)		Wind Speed (mph)	
	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
Class room	22±0.6	30.7±0.9	55.0±1.2	37.5±9.1	18.6±0.5	14.0±4.2	14.5±0.01	14.6±0.02	1.7±0.1	1.7±0.5
Laboratory	21.8±0.6	30.2±0.5	59.9±3.2	39.2±7.4	19.9±0.6	13.9±2.5	14.5±0.03	14.6±0.0	1.1±0.2	2.6±0.7
Office	21.2±0.4	30.1±0.3	53.8±2.4	32.1±0.9	18.3±0.9	14.3±2.3	14.6± 0.0	14.6±0.03	1.1±0.3	2± 0.8
Canteen	21.7±0.3	32±1.1	38.9±9.7	56.8±1.9	13.1±5.1	22.3±1.4	14.6±0.03	14.6±0.03	1.3±0.1	1.0± 0.2
Common room	21.3±0.4	31.6±1.4	28.2±2.3	57.4±2.5	8.0±1.7	22.0±1.0	14.7±0.1	14.6±0.0	2.2±0.6	2±1.0
Library	21.2±0.4	31.7±1.1	40.2±1.3	47.9±1.7	13.5±0.9	19.2±0.6	14.6±0.0	14.5±0.03	2.7±0.7	1.4±0.4
Computer lab	21.3±0.3	29.1±1.4	38.4±7.6	39.5±8.5	13.5±2.5	12.4±3.3	14.6±0.03	14.6±0.03	1.4±0.3	0.9±0.1

Table 5: Relative abundance of fungi (mold and yeast) in indoor environment.

Organisms	Relative abundance
Mold	
<i>Alternaria</i>	0.7%
<i>Aspergillus</i>	22.3%
<i>Cladosporium</i>	30.7%
<i>Fusarium</i>	0.03%
<i>Penicillium</i>	22%
<i>Stachybotrys</i>	2.6%
Yeast	
<i>Candida</i>	21%
<i>Rhodoturolo</i>	0.6%

Bacteria presence in respect to area indicated that Gram-positive bacilli and Gram-negative short rods were dominant in university indoor environment as they were present in all sampling sites except computer lab (Table 6). Coccobacilli and diptheroid rods were another dominant group found in offices, canteens and computer labs. Diplococci and bunches were found in 2 sampling sites only. Offices are the places where maximum seven varieties of bacteria were found. Fungal presence indicated that *Aspergillus*, *Penicillium*, *Candida* and *Rhodoturolo* were dominant and present in all seven sampling sites (Table 6). *Cladosporium* was isolated from six sampling sites. *Stachybotrys* was present in offices, canteens and computer labs. *Alternaria* was found in laboratories and offices while *Fusarium* was present in computer labs only. According to WHO standard, *Aspergillus* and *Stachybotrys* species are not allowed in indoor environment [6]. Their presence in sampling sites might be harmful for human occupants.

Statistical analysis by two sample *t*-test ($p = 0.05$) indicated that there were significant differences in mean temperatures ($p = 0.00$), bacterial counts ($p = 0.002$) and fungal counts ($p = 0.035$) in winter and summer seasons. Mean bacterial and fungal counts were higher in summer (205 ± 39 and 306 ± 102 CFU.m⁻³) compare to winter season (133 ± 26 and 199 ± 59 CFU.m⁻³). Temperature might be a reason for this elevated count as mean temperature was high in summer ($30.8 \pm 0.4^\circ\text{C}$), while it was $21.5 \pm 0.1^\circ\text{C}$ in winter. There was no significant difference in wind speed, barometric pressure, dew point and humidity ($p > 0.05$). Correlation analysis by Pearson's correlation test ($p < 0.05$) indicated that there were significant correlations between bacterial and fungal count in summer ($r = 0.802$, $p = 0.030$), wind speed and fungal count in summer ($r = 0.77$, $p = 0.043$) and between humidity and fungal count in winter ($r = 0.79$, $p = 0.03$). These results were consistent with previous studies where a relationship was reported between dampness and microbial count [9-10]. Not only fungal growth Adams et al. [11], but bacterial growth can also be higher in moist environment [12]. Several fungal genera have been reported in indoor environment including *Acremonium*, *Alternaria*, *Aspergillus*, *Chaetomium*, *Cladosporium*, *Mucor*, *Paecilomyces*, *Penicillium*, *Stachybotrys*, *Ulocladium* and *Verticillium* [40,-42].

Previous epidemiological studies showed that the dampness is associated with increase rate of respiratory illnesses including cough, asthma, bronchitis and eczema [12]. Poor indoor air quality can have resulted in less productivity of occupants [43-44]. Toxic reactions, hypersensitivity pneumonitis and other health effects may occur due to the exposure to airborne microorganisms containing aerosol [38]. Additionally, moisture content and humidity can determine the level and type of microorganisms in indoor environment.

Conclusion

In the current study, the presence of elevated concentration of fungi and its significant relationship with moisture in indoor environment indicates that the occupants of these indoor places in university premises are at a risk of respiratory infections. They include students, teaching faculty, and office staff who work for long hours in university. The potential of indoor microorganism especially the molds to cause infections indicates that there is a need of detailed epidemiological study and both indoor and outdoor air quality monitoring in the city and educational institutions with high public occupancy. Furthermore, there is a need of indoor air quality management for the prevention of epidemic of respiratory diseases in occupants of indoor environment.

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