Qualitative and quantitative evaluation of bioaerosol in selected public spaces

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Abstract

This study is aimed at quantitative assessment of air borne bioaerosol in public spaces. Indoor air quality is an important contributor because modern human beings spend most of their life time in indoor. Therefore the quantification of airborne bacteria and fungi in public spaces such as health centers, auditoriums, libraries, seminar halls and rest room/toilets in Bangalore university campus was carried out by following standard impaction method and reported as colony forming units per cubic meter of air (CFU/m^3). The results of above study revealed that average total fungal count in health center was 1,256 CFU/m^3 in air conditioned (A/c) Auditorium; it was measured as 175 CFU/$m^{3,}$ at in library 2523 CFU/m^3 , in seminar Hall 395 CFU/$m^{3.}$, in Toilets it was 1,416 CFU/$m^{3.}$. The average Total bacterial bioaerosol measured at health center was of 654 CFU/m^3, at air conditioned Auditorium (AC) it was 310 CFU/m^3, in library 1,159 CFU/m^3, in seminar hall 586 CFU/m^3 and in toilet 1,438 CFU/m^3 respectively. The indoor to outdoor ratio of fungal and bacterial aerosol were found to be equal to 0.005 or > 0.005. From the above study we can conclude that the bioaerosol concentration in indoor environment is comparatively less than outdoor but high enough to cause infection in indoor occupants.

Keywords — *Indoor public spaces, bacterial aerosol, fungal aerosol, Indoor environmental conditions.*

I. INTRODUCTION

Indoor Air pollution is now a global concern. Bioaerosol is an important contributor for indoor air pollution, as they can be pathogenic and cause an allergic reaction following inhalation. Bioaerosol are composed of micro-particle which may contain alive or dead bacteria, molds and dampness, pollen etc., prolonged inhalation of such elements can be the cause of Asthma and allergic reactions.

According to Global Asthma Report [1] asthma kills 1000 people every day and effects as many as 339 million people and prevalence is rising in low and middle income countries. They disproportionately suffer in most severe cases. An Indian study on Epidemiology of Asthma, respiratory symptoms and chronic Bronchitis [2] reports that, 17.25 million Indians suffer from Asthma. People in metropolitan cities are facing different pulmonary problems during the onset of different seasons. Bengaluru being a metropolitan city has become a hub of allergic patients, as it has a prevalence of asthma at 9%, 10.5%, 18.5%, 24.5% and 29.5% during the year 1979,1984,1986,1994 and 1999 respectively [3] . The present scenario is much more serious due to the exponential growth of the city vertically as well as poor air quality, urbanization and urban sprawl.

Buildings in urban areas have undergone radical changes over past few decades, thereby resulting in less opportunity to exchange indoor air with outdoor air. Environment Protection Agency [4] Guidelines for Indoor air quality indicates that, indoor levels of pollutants may be 2-5 times and occasionally more than 100 times higher than outdoor levels. Wold health organization [5] reports that sick building syndrome complaints are related to poor indoor air quality. Sick building syndrome (SBS) it is a sickness where people in a building suffer from symptoms of illness or feel unwell. The symptoms tend to increase in severity with the time people spend in the buildings and improve over time or even disappear when people are away from the building. The main symptoms are headache, eye, nose and throat irritation, fatigue, dizziness and nausea. Indoor environment is thought to be the principal factor in contributing to the build-up and spread of airborne microbial contamination and toxin.

Houses, work place and office buildings in metropolitan cities found to contains elevated levels of bioaerosol and endotoxin due to the presence open solid waste dump, composting units and public bathrooms, toilets, manufacturing units, paint shops, dairy industries [6] sewage treatment plants, swimming pools, cotton mills , grain storage , processing buildings , and animal house near vicinity. The long time exposure is associated with abnormal health issues in building occupants.

In the present study Assessment of indoor bacterial aerosol and fugal aerosol concentration was conducted in a few selected indoor public spaces such as health centre, auditoriums, libraries, seminar halls and rest room/toilets in Bangalore university campus. It has become very important to identify different risk factors and establish exposure threshold

limit for microbial and chemical pollutants. Characterizing and quantifying the pollutants help in taking appropriate measures to control pollutants which are of biological origin.

II. METHODOLOGY

A. Study area: Location map of study area

Fig: 1 sampling location image of Jnana Bharathi Campus

B. Geographical Area and Sampling site

Bangalore University is one of the largest universities in South-Asia: Jnana Bharathi (JB) Campus. Jnana Bharathi campus of Bangalore University is located in the western side of Bangalore city and spreads over in 1040 acres. It lies between 12˚55'59" & 12˚57'33" latitude and lies between 77˚29'45" & 77˚31'12" longitude Fig.1. There are around 131 buildings in the campus comprising the roof top area about 97,850 sq meters. There are 29 buildings which are more than 1000 sq meters; 92 buildings less than 500 sq meters; 10 buildings are between 500 to 1000sqmts. All put together it covers area of 24.18 acres.

Assessment study of indoor bacterial aerosol and fungal aerosol concentration was carried out at health centres, auditoriums, libraries, seminar halls and rest room/toilets in Bangalore university campus. The specification of dimension of study area in Bangalore university campus is given in Table I. All Indoor sampling areas are naturally ventilated except Auditorium which is air conditioned room. It does not contain any ventilation window and the auditorium hall is designed with acoustics, it has two entries and exit doors. Wall mounted fans and few table fans are provided for forced ventilation.

TABLE I

Details of the sampling location, dimension and air exchange indoor space

Sr. No	Sampling site	sampling area dimension	Volume of air sampled in cubic meter	Rate of air exchange in Air change/hr
1	Health centre	3m X 3.6m	32	9
2	Auditorium(AC)	24m X 8m	4608	13
3	Library	36m X 22m	15840	11
4	Seminar hall	3m X 9m	270	8
5	Toilets	3m X 2.4m	64	9.8

C. Measurement of Indoor environmental conditions

Indoor Air Sampling was carried out following ASTM (2014) E1370-14 [7]. Preliminary examination of sampling sites was carried out and standard temperature, relative humidity, carbon dioxide and carbon monoxide concentration was measured using wet and dry bulb thermometer and expressed in degree Celsius, relative humidity was expressed in percentage, oxides of carbon were measured using digital CO_2 and CO meter and expressed in part per million (ppm) levels.

D. Sampling and Enumeration of Indoor bacterial bioaerosol

Indoor air samples were collected from health centres, auditoriums, libraries, seminar halls and rest room/toilets at Bangalore university campus. Indoor bacterial and fungal aerosols sampling was carried out following methods prescribed by Anderson (1958) [8] "New sampler for the collection, sizing, and enumeration of viable airborne particles". Anderson single stage Microbial air Sampler - HiMedia (LA474) was used to collect indoor air sample of bacteria by adjusting the flow rate to 28.7 L/min and sampled for 1 min on sterile **Nutrient** media, Selective, enrichment and differential media (HiMedia). Before each sample is collected, sampler was wiped with cotton dipped in isopropyl alcohol [9] Samples were taken in duplicate and a field bank was maintained at all sapling sites.

Samples were collected in different days during winter (December to February), summer (March to May), monsoon (June to September) post monsoon seasons (October to November) of the year during working hours in between 10AM to 12.30PM at all sampling sites. One set of outdoor samples were collected at all sampling location. [10] During December 2017 to November 2018.

At each sampling sites 12 indoor samples were collected and one outdoor air sample was collected. sampled plates were brought to microbiological laboratory in ice box and incubated in dark room at different temperatures namely 30 ± 2°C for minimum 3 days and at 55± 2°C minimum 7 days

Enumerations of bacterial organisms were carried out following APHA, (2012) [11] by Standard plate count (viable count) method using Digital microbial colony counter (Model DCC100) and reported as colony forming units (CFUs).

E. Identification of bacterial aerosol

Bacterial isolates were characterized and identified by cultural, morphological and microscopic examinations. Different biochemical tests such as Gram staining, sugar fermentation, Indole , Methyl-red, Voges-proskauer, citrate, catalase test, Oxidase, Triple sugar iron test and test were employed to identify the bacterial isolates following the method described in Bergey's manual of Systematic bacteriology (2009)[12].

F. Isolation and enumeration of fungal aerosol

Fungal aerosols were collected on sterile Rose Bengal agar medium and Potato Dextrose agar medium containing Petri plats by impaction method. Samples were collected in triplicates. Laboratory media blank and field blank is maintained at all sampling sites. After the sampling the sample plates were aseptically brought to laboratory and incubated at 25 °C and 37 °C for 7 days for moulds growth. Since fungi growth is the highest at 37 °C, while filamentous fungi growth is best at 25 °C, both these temperature were included in the studies. Fungal growth was observed as colony and were counted using colony counter. Total number of fungal colony appeared on the surface of the grid or squares and Total fungal colony count are reported as CFU/m^3 after enumeration. Identification of fungal isolates was carried out by light microscopy observation of fungal morphology by following standard methods [13], [14].

III. RESULT AND DISCUSSION

A. Indoor environmental factors

The Indoor environmental condition measured during sampling was recorded. The average temperature, relative humidity, carbon dioxide and carbon monoxide measured (Table-II) in Health centre and recorded as 26.7°C, 56.8%, 513ppm and 1ppm respectively. Similarly, temperature, relative humidity, carbon dioxide and carbon monoxide in Auditorium (AC) was measured as 26.5°C, 67.3%, 505 ppm and 1ppm respectively. In the Library, temperature, relative humidity, carbon dioxide and carbon monoxide was measured as 25.8°C, 66.5%, 540ppm and 0ppm respectively. In the Seminar hall , temperature, relative humidity, carbon dioxide and carbon monoxide was measured as 27.25°C, 55.5%, 406ppm and 0ppm respectively. In toilets, temperature, relative humidity, carbon dioxide and carbon monoxide was measured as 26.17°C, 47.2%, 538ppm and 0ppm respectively. The temperature and relative humidity recorded at the sampling sites were found to be higher than the WHO prescribed (WHO, 2010) standard namely temperature range of 18-26˚C and relative humidity of the range of 40-70%. Carbon dioxide levels in the range of 350-1000ppm and carbon monoxide levels of 0-9ppm were found to be within the prescribed limit (WHO, 2010). Average concentration of bacterial and fungal concentration in 5 different indoor environments is summarized in Table-2.

TABLE II

Average Bioaerosol concentrations in different indoor sampling sties

Sampling sites	Average bioaerosol concentration CFU/m^3			Microclimatic conditions		
	Total Fungus	Total Bacteria	Avg. Temp (°C)	Avg. RH (%)	Avg.CO (ppm)	Avg.CO_2 (ppm)
Health centre	1,256	654	26.7	56.8	1	513
Auditorium (A/c)	175	310	26.5	67.3	1	505
Library	2,523	1,159	25.8	66.5	0	540
Seminar hall	395	586	27.25	55.5	0	406
Toilet	1,416	1,438	26.17	47.2	0	538
Outdoor	38,975	27,895	28.9	48.9	1	589

B. Indoor Fungal aerosol

The average number of fungal aerosol measured at health centre was 1,256 CFU/m^3; the fungal isolates of health centre identified were belonging to gene to Aspergillus and Alternaria. The higher number of fungal aerosol inside health centre are attributed to the number of visitors, patients and their accompanying people contributing to culture and propagation of these microbes. Constant movement of patient inside the dressing room for treatments and for minor wound dressing and other related activities aerosolize the dust containing fungal spores. The room inside the health centre is provided with big ventilations which contribute to indoor bioaerosol rush from outdoor air. Number of fungi found in indoor environment is high enough to cause respiratory and asthmatic problems upon prolonged exposure show in the Fig.2.

Similarly, [15] states in there finding that, indoor air in nursing homes contain Alternaria alternata, these fungus emit allergens which contribute to respiratory problem. Hence hospital and nursing home environment require more attention to indoor air quality.

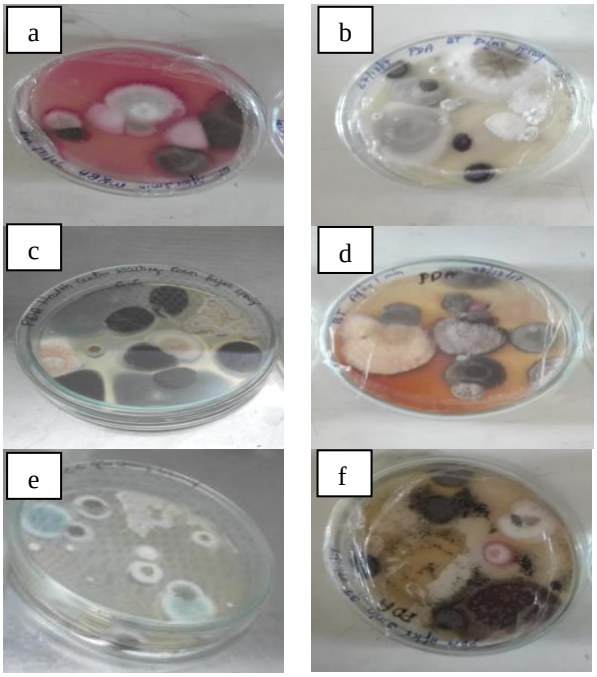

Fig.2: Fungal sample of a) health centre b) auditorium (A/C) c) library d) seminar hall e) toilets f) outdoor

The auditorium (A/C) and seminar hall (non A/C) fungal aerosol was measured as 175CFU/m3 and 395 CFU/m3.Fungal isolates identified were belonging to genera Cladosporium, Alternaria and Penicillium were predominated in air conditioned auditorium. The sources of air borne fungal contamination in air conditioned auditorium was attributed to landscape which were covered with velvet kind of material and acoustic materials on the walls which gave enough surface area for proliferation of fungal colonies [16]

Other sources of fungal aerosol might be, leaks in wall embedded water circulation pipes which lead to water soaking to porous material such as ceiling boards and partition wall. Airborne spores were transmitted by the air conditioning system to the whole building causing the widespread of the mould growth. Even though work space and auditoriums had increasingly innovative air conditioning system and air filters, the mold based incidents had grown steadily. similarly reported by [17] and conclude that" Instead of providing essential good indoor air to the occupants, air-conditioning systems have become 'highway' for deadly disease".

In library average number indoor fungal bioaerosol measured as 2523 CFU/m3. Fungal genera identified in library were *Alternaria*, *Aspergillus* and *Cladosporium*. The number of fungal aerosol was significantly high in library. The library had materials like books, maps, drawings paper or parchment documents, wood, textile, leather and plastic materials. These materials contained cellulose, lignin, natural glues proteins and sugars which might act as carbon source for airborne fungal and bacterial aerosol result in high microbial population in library area. Generally, the low temperature and high relative humidity in library greatly contributed to bio degradation of books. Cellulose and dust particles provide surface area for proliferation of fungal biomass. Similar finding had been reported on fungal concentration in library by [18] in indoor environment JNU library. Air borne viable fungi aerosol measured inside toilets as 1716 CFU/m^3 and fungal genera like *Aspergillus. Candida and Penicillium* were identified in indoor air of the toilets. This might be because of inflow and outflow of air containing fungal spores and insufficient light penetration lowered the natural killing process by light. The vegetation cover outside the building carries fungal load inside the toilet as the outdoor airborne fungal concentration of Bangalore university campus measured as 38,976 CFU/m3. These fungal could cause local infections and allergies among the immune suppressed person such as aged ones and children. Fungi also produce allergens called Mycotoxin which cause serious health problems in human. [19, 20]

C. Indoor Bacterial aerosol

The average concentration of viable indoor bacterial aerosol measured at health center as 654CFU/m^3 bacteria identified *Pseudomonas sp.*, *Staphylococcus sp.* and *Klebsiella sp.* The sources of the viable bacterial in indoor air may be attributed to infection carrying patients and other visitors accompanying them. Other sources may be due to improper disposal of medical waste that contains blood which are kept uncovered inside and out of health center can become a potential source. The microorganisms in hospital environment are primary agents of nosocomial infections; large numbers of pathogenic microorganisms are always presents in the hospital atmosphere during bronchoscopy examination and these bioaerosol require 95 minutes time to reach background concentration [21] and may cause serious health hazards so it is important that the hospital ambient air should be continuously sanitized.

The average concentration of viable indoor bacterial aerosol at auditorium (A/c) and Seminar Hall (non A/C) was measured as 310 CFU/m^3 and 586 CFU/m^3.Bacterial isolates identified as *Micrococcus sp.*, *Staphylococcus sp. and Bacillus sp.*. The bioaerosol number is high due to proliferation of bacteria with in HEPA Filter of air conditioning systems. The HEPA filter can filter 0.3um size particles in the air. These particles include viable bacterial which get enough surface area for their proliferation in HEPA filter. .Further these slowly get released into the indoor air resulting in increased bioaerosol concentration.

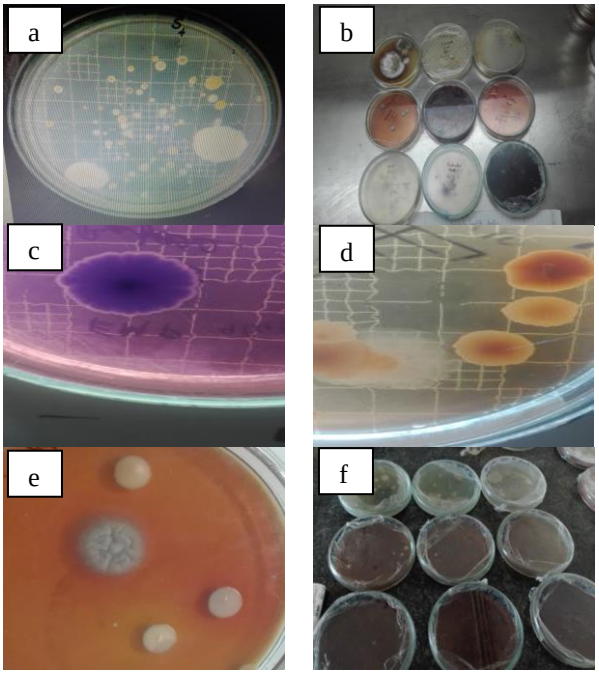

Fig 2 Bacterial Sample Plates of a) Health Centre b) Auditorium (A/c) c) Library d) Seminar Hall e) Toilets f) Outdoor

In library indoor bacterial aerosol measured as 1,159 CFU/m3.Bacteria identified were *Klebsiella sp.* and *Staphylococcus sp.* Higher bacterial counts were primarily attributed to the number of library visitors, Paper parchment and the rate of shedding of human skin cells who spends many hours reading and from library workers. Bacteria may release infectious microbes from human respiratory system on sneezing, talking and other related activity. Aerosolization of settled floor dust and improper cleanliness may have influence on indoor bacterial concentration. [22]

In toilets the average number of bacterial aerosol measured as 1,438 CFU/m^3.bacterial isolates identified and in toilets there were *E.Coli. Streptococcus sp., Enterococcus sp and Bacillus sp.* The high microbial load in toilets are attributed to high influx of people on daily basis and the behaviour of toilet users and indoor air quality within the toilet, the activities such as flushing the toilet properly, littering the toilet floor etc. Human population and their activities affect the concentration of bacteria which are released during brisk movements like talking, coughing and sneezing etc., The presence of the high number of visitors, wetness of toilet floors, lack of litter bins and refuse lids and lack of ownership of in public toilets results in the increase in airborne microbial load. The fecal contaminated surfaces such as the toilet seat, flush handle, restroom floor etc., are potential sources for infection. [23]. The outdoor environment of Bangalore university campus number of bioaerosol measured as 27,895 CFU/m^3 .similar finding was reported by[24] Sivasakthivel S & Nandini N., (2017)

TABLE: III

Indoor - to-outdoor ratio of bioaerosol concentration

	Sampling sites	Fungal	Bacterial
I/O Ratio	Health centre	0.02	0.019
	Auditorium (AC)	0.004	0.011
	Library	0.05	0.04
	Seminar hall	0.007	0.017
	Toilets	0.04	0.019

I/O Ratio: Indoor - to-outdoor Ratio

The indoor to outdoor Ratio bioaerosol concentration was calculated was found to 0.05 or < 0.05 for fungal and bacterial aerosol. This revels that outdoor bacterial and fungal pollution was one of the highest source of indoor bioaerosol pollution. Similar findings are reported by [16]

CONCLUSION

From the above study, it can be concluded that bioaerosol concentration in indoor is comparatively low when compared to outdoor concentration. The average temperature recorded in Bangalore is always high in summer (January to April) months and it is considered as dry period. The temperature in summer may reach as high as 39˚C and relative humidity measured as 42% in midsummer .Similarly Indoor average relative humidity measured at sampling sites during summer was 47% and temperature recorded as 28˚C, the lowering of temperature may be attributed to enclosed space and restricted light penetration. During summer seasons the soil bone microbes and microbes present on leaf surface dominate the indoor air. The fungal spores belonging to genera *Alternaria spp., Aspergillus spp.,* and *Cladosporium spp.* were found in higher concentration in indoor sampling sites.

The variability in bacterial community structure could not be predicted by meteorological conditions other than temperature and relative humidity. Local terrestrial sources such as water bodies, vegetation cover, and waste dump sites, presence of waste water treatment plants, and flowing of waste water polluted river Vrushabhavathi inside the Bangalore university campus play greater role in structuring the airborne communities than short duration shift in the atmospheric conditions. The camps is also has a reach vegetation cover sourcing the outdoor fungal concentration. The bioaerosol concentration in breathing zone of the indoor environment is contributed by different sources and factors. Apportionment of indoor airborne microbes depends on primary sources such as humans, pets, plants, HVAC systems and plumbing activity and secondary source such as building infrastructure.[25] The hospital indoor environment contain moderate to

heavy levels of fungal and bacterial aerosol which is significantly sourced by patients and visitors; other secondary sources of airborne microbes in indoor sampling sites were attributed to carpets dust, floor dust and settled dust on walls, mould growth on walls, presence of books and equipments. Bacterial species identified during study such as *E.Coli. Streptococcus sp., Enterococcus sp. and Bacillus sp* are all contributed by human micro flora. Indoor air of health centre and library had shown the presence of *Legionella sp.* and *Klebsiella spp.*, which may become major cause for nosocomial infection in library and hospital visitors and employs, the concentration vary significant in day time than in night time [26]. However, these can pose serious health problems as the air circulation and light penetration inside the indoor environment is less and makes the indoor air biologically polluted. However [27] reports that bacteria which are originated from cow dung effectively bioremediates hydrocarbons and benzene

In Bangalore outdoor temperature range recorded in winter (December-February) was 12-28˚C and relative humidity as 23-100% similarly in monsoon (June- September) temperature recorded was 20-28˚C and relative humidity 31-100%. In post monsoon (October-November) temperature range were 13.8-32˚C and relative humidity 25-100%. Similarly indoor average temperatures range at sampling sites was found to be 25- 27˚C with higher average relative humidity 55-66% during sampling period. A higher relative humidity condition makes air heavier and enhances stagnation of the air inside the room giving less chances of fresh air exchange, results in increasing indoor bacterial aerosol. Higher bacterial airborne contamination increases the chances of acquiring nosocomial infections and allergic disorder in immune depressant occupants such as children and elderly people. The Indoor to outdoor ratio of bioaerosol indicates that the concentration of outdoor bioaerosol is high and indoor bioaerosol is less. However, indoor environment conditions such as temperature, relative humidity, carbon dioxide and carbon monoxide would influence pollution levels and affect the occupant's health upon prolonged exposure. Hence it is highly recommended to maintain personal hygiene and clean environment conditions in toilets, periodic cleaning of furniture, equipments and carpets and book racks in the library which forms a secondary sources for the generation of bioaerosol. The HAPA filters need to periodically change as it can become major sources of fungal spores in Air conditioned indoor environment.

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Acknowledgment

The authors wish to acknowledge the contribution of Dr. Nandini, Professor and Chairperson, Department of Environmental Science, Bangalore University, Bengaluru.

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