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### Study of bio-aerosols in a prominent temple in Mumbai City, India

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Numerous health complaints occur with respect to indoor air quality and scanty information is available on the air flora, particularly with respect to bio-aerosols in India. This paper reports a study which examined air quality in a prominent temple in the city of Mumbai. It was found that the indoor microbial load was significantly higher probably because appropriate ventilation systems were lacking. In the festive month of September, the highest bacterial counts ( $13.98 \times 10^4$  CFU/m<sup>3</sup>) and fungal counts ( $9.22 \times 10^4$  CFU/m<sup>3</sup>) were observed. *Pseudomonas* spp. and *Aspergillus* spp. were found to be predominant in the air microflora sampled. Correlation analysis with meteorological factors such as temperature, relative humidity and wind speed showed that these were in a given area are largely responsible for the variation in microbial loads observed. The data generated underline the question of monitoring crowded areas including places of religious importance. Some remedial measures are suggested.

Keywords: bio-aerosols; indoor; outdoor; bacterial; fungal; meteorological factors

#### Introduction

India is a tropical country and its climate is not constant. The climate in any region also signifies the composition of microbes existing within that environment. India's rapid population growth, industrialization and urbanization are adversely affecting the environment. Because of overcrowding and poor ventilation, people are exposed to various pollutants micro-organisms leading to an increase in respiratory disorders. In general, indoor microflora concentrations of a healthy work environment should be lower than the outdoor concentrations at the same location [1]. Each cubic metre of indoor or outdoor air may contain thousands or even millions of micro-organisms and biological particles consisting of droplets or particles ranging from 0.5 to 30  $\mu$ m, collectively referred to as bio-aerosols [2]. Relatively high levels of one type of airborne microbe may represent very low risks, while extremely low levels of more infectious contaminants could trigger potential health problems.

There have been studies worldwide of bio-aerosols; epidemiological and toxicological studies have provided further evidence of a possible link between bio-aerosols and

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sick-building syndrome [3]. The most common micro-organisms found indoors are fungi and bacteria [1]. Our study compares indoor and outdoor air quality in terms of its microbial load so as to generate baseline data, and we also suggest some remedial measures. The sampling location selected for the study is a prominent religious place in an extremely crowded area of India's fastest growing city, Mumbai. The study is one of the first attempts to characterize the microbial load in a religious place in Mumbai and attempts to correlate its significance to health and hygiene.

#### Materials and methods

#### Sampling site

The financial capital of the country, Mumbai (latitude 18°55'N, longitude 72°54'E) has an ever increasing population and the number of devotees visiting prominent temples has been swelling substantially. The study therefore aimed at collection of air samples from one such popular and frequently visited temple in Mumbai's central suburbs, the Shri. Siddhivinayak temple. The climate in the city usually remains sultry and humid through the year due to its close proximity to the Arabian Sea. Temperatures range between 27 and 33 °C in the summer months (March–May), between 24 and 30 °C (June–September) during rains and between 22 and 29 °C in the winter months (October–February). This area was particularly chosen for sampling since it is not only a densely populated site with an intense vehicular traffic problem but is also perpetually affected by the floating population of devotees. The characteristic feature of the site was that the number of people there was greater than that at any other location in its vicinity. The rate of influx of people was counted manually and was on an average found to be approximately 1200–1600 people per hour; a figure that increased to  $\geq 2000$  people during festivals. This may be one of the key factors affecting the bio-aerosol concentration at this particular site.

Ambient air sampling was carried out simultaneously within indoor and outdoor premises of the temple. For the indoor study, the sampling site was chosen close to an area where devotees spend the time meditating or performing rituals and the samplers were placed at a height of 1.2 m from the ground level. For the outdoor study, the samplers were placed closed to the temple exit, about 20–25 m away from the traffic.

#### Sampling method

Sampling was carried out from July 2007 to November 2007, i.e. for a period of over two seasons (peak monsoon and early winter), twice a day (morning and afternoon), and twice a month. Sampling was carried out in duplicates unless otherwise mentioned. A National Environmental Engineering Research Institute Low Volume Air Sampler was used in the study. The sampler was a portable battery-operated instrument capable of achieving a maximum flow rate of 0.25 L/min. The air released from the outlet was made to impact on standard 90 mm sterile Petri plates containing an appropriate sterile culture medium. The air samples were also bubbled through sterile liquid impingers dipped in sterile phosphate-buffered saline (PBS). This PBS was diluted appropriately and plated on Nutrient agar and Sabouraud's agar for bacterial and fungal growth analysis [4]. Impaction was carried out at the maximum flow rate for a time period of 30 min on Nutrient agar for bacteria and Sabouraud's agar for fungi. The sampling air flow rate was standardized, preset to 0.25 L/min and maintained constant throughout the study.

The plates were incubated at room temperature and 37 °C for 24–48 h and up to 72 h for favourable bacterial and fungal growth, respectively. Colonies on the agar plates were counted and the bacterial and fungal concentrations in air were calculated using a standard formula and expressed as colony-forming units per cubic metre (CFU/m<sup>3</sup>).

$$CFU/m^3 = CFU \times 1000/V_s$$

where,  $V_s$  is the air volume in litres passed through the sampler [5,6].

Metrological factors were taken into consideration and temperature, relative humidity (RH) and wind speed were also recorded at the time of sampling. Table 1 summarizes the climate conditions during the study period.

#### Taxonomic identification

Different bacteria and fungi thus isolated were subjected to further characterization and identification. All the procedures were carried out under aseptic conditions during analysis. Isolates were identified by standard biochemical tests including IMViC test (Indole Methyl red, Voges Prosauker, Simmon Citrate), and sugar use tests (for Arabinose, Glucose, Mannitol, Myoinositol, Xylose), etc. The organisms were also tested for catalase production and motility. Bacteria were identified up to genus level and a few up to species level using *Bergey's Manual of Determinative Bacteriology* [7–9]. Fungal isolates were mainly studied on the basis of macro-morphological features using lactophenol Cotton Blue [4,10] and identified with the help of standard microbiological books and literature available on mycology [11].

#### Statistical analysis

The One-Way Analysis of Variance test was used to ascertain the distribution of total bacterial/fungal count across the five months of study and comparative analysis of bacterial vs. fungal count, indoor vs. outdoor microbial load as well as total microbial count across the period of study. The findings were graphically represented as Mean  $\pm$  Standard Error Mean. The relationship between total microbial counts and each of the meteorological factors affecting the study was examined by Spearman correlation analysis. In all cases,  $p \le 0.05$  was considered significant. Statistics were assessed and graphs were plotted using GraphPad Prism (ver. 5.0) Software (GraphPad Software Inc.).

	Temperature (°C)		Relative humidity (%)		Wind speed (km/hr)	
	Min	Max	Min	Max	Min	Max
July	24	30	72	97	6	30
August	24	30.4	69	97	6	24
September	25	32.8	61	95	6	16
October	24.4	36.2	23	97	6	12
November	23.4	35.8	26	75	4	12

Table 1. Monthly maximum and minimum values of basic meteorological factors during the study period.

#### **Results and discussion**

This study was carried out with an aim of evaluating bio-aerosol concentration in the air of a densely populated religious place of worship located in the heart of a major metropolitan city, Mumbai. A total of 160 air samples were collected for both bacterial and fungal analysis from July to November. The total microbial count ranged between  $11.45 \times 10^4$  to  $23.20 \times 10^4$  CFU/m<sup>3</sup> with the highest being in the month of September and significant p = 0.0355 increase being observed in microbial concentration from August to September (Figure 1, Table 5). This could be attributed to the large number of people visiting the temple in September, because of the festive season. In this month, very large numbers of devotees come to the temple during the 10-day festival of Ganesh, known as 'Ganeshot-sav'. Earlier reports have shown that the concentration of airborne micro-organisms and number of visitors in a given closed environment are positively correlated [12]. The average bacterial and fungal counts were found to be  $9.68 \times 10^4$  and  $7.25 \times 10^4$  CFU/m<sup>3</sup>, respectively, for the period during the study.

Airborne bacterial concentration from July to November ranged from  $6.39 \times 10^4$  to  $13.98 \times 10^4$  CFU/m<sup>3</sup>. It can be observed from Figure 2, Table 4 that a significant reduction in bacterial load was found between indoor afternoon and outdoor morning in July (p = 0.0004) and August (p = 0.0029). But, maximal changes in bacterial load with change in day time were found during the month of September. A statistically significant decrease (p = 0.0003) in the bacterial count from morning to afternoon was observed both indoors and outdoors. Airborne microbial quality and quantity is bound to vary with the time of day [13]. The decrease in microbial population from morning to afternoon may be caused by the establishment of convective air currents raising the aerosol particles from ground/ floor surface with rising of the sun [14]. Although bacterial counts in the morning



Figure 1. Represents distribution of total microbial count in CFU/m<sup>3</sup> in each of the months included in the study. Results are expressed as the mean  $\pm$  SEM for month. Evaluation of the significance of differences between the means of parameters at different time points was performed using the One-way ANOVA. In all cases  $P \le 0.05$  was considered significant.



Figure 2. (A–E) Represents distribution of bacterial count in CFU/m<sup>3</sup> at the two sites within temple premises taken at two different time points in each of the months included in the study. Results are expressed as the mean  $\pm$  SEM for each group. Evaluation of the significance of differences between the means of parameters at different time points was performed using the One-way ANOVA. In all cases  $P \le 0.05$  was considered significant.

continued to be higher than those in the afternoon in the months of October and November as well, the difference was not statistically significant.

A total of 21 different bacterial isolates were recovered from the sampling site in this study. Two isolates were tentatively identified up to genus level and six isolates up to species level (Table 2) based on their Gram nature, biochemical tests and sugar fermentation tests; 13 isolates remain unidentified. Although most aerobiological studies carried out in India have reported a higher concentration of Gram-positive bacteria than Gram negative [14–16], in our study it was observed that the majority of bacteria were Gram negative. These findings are similar to those reported by Pathak and Verma [17].

Bacteria identified up to species level included *Citrobacter diversus*, *Pseudomonas fluorescens*, *Shigella sonnei* and *Serratia marcescens* (Table 2). The presence of *Pseudomonas* spp. in air has been very commonly reported in similar studies previously carried out in vegetable markets [17], food godowns [18], animal sheds [19], hospital wards [16], railway stations and even public gardens [15]. Presence of organisms such as *C. diversus* (uropathogen) [20] and *S. sonnei* (which typically spread via faecal-oral route) [21] is a matter of concern. Poor hygiene and sanitation in the periphery of the sampling site probably explain the presence of these. *Klebsiella pneumonia* sub spp. *ozaenae*, a diarrhoeagenic species [22] of *K. pneumonia* was also identified. Another uncommon bacterial isolate found was *Vibrio cincinatiensis*, a Gram-negative non-spore-forming rod known to cause meningitis [23, 24]. As it is difficult to ascertain the occurrence of such rare organisms only on the basis of classical microbiological techniques, there is a need for molecular methods such 16s rRNA gene sequencing for further confirmation [24].

Average fungal counts ranged between  $5.06 \times 10^4$  and  $9.22 \times 10^4$  CFU/m<sup>3</sup>. Unlike bacterial load, changes in fungal load remained statistically insignificant in the months of July and August rather than in October and November (Figure 3). Although a statistically signifi-

Name of the test	Citrobacter diversus	Pseudomonas fluorescens	Shigella sonnei	Klebsiella pneumonia sub spp. ozaenae	Serratia marcescens	Pseudomonas spp.	Vibrio cincinnatiensis
Gram nature	_	_	_	_	_	_	+
Indole	+	_	_	_	_	_	_
MR	+	+	+	+	+	_	_
VP	_	_	_	_	_	_	_
TSI							
Slant	А	K	Κ	А	K	Κ	А
Butt	А	А	А	А	А	Κ	А
Gas	+	_	_	_	_	_	_
$H_2S$	+	_	_	_	_	_	-
Citrate	+	+	+	_	+	+	_
Motility	+	+	_	_	+	+	+
Catalase	+	+	+	+	+	+	+
Sugars							
Lactose	+	_	_	+	_	_	_
Glucose	+	+	+	+	+	_	+
Mannitol	+	_	+	+	+	_	+
Arabinose	+	+	+	+	_	ND	+
Myo-inositol	_	_	_	_	+	ND	+
Xylose	+	+	_	+	+	ND	+

Table 2. Biochemical characteristics of bacterial isolates.

'+', positive; '-', negative; K, alkaline; A, acidic; ND, not done.

cant increase in load -p = 0.0109 and p = 0.0058 (Table 4) was found from morning to afternoon in the outdoor premises of the temple in the months of October and November, respectively, maximal changes in the load were observed in September. Total fungal concentration was found to be highest indoors in the morning with statistically significant



Figure 3. (A–E) Represents distribution of fungal count in CFU/m<sup>3</sup> at the two sites within temple premises taken at two different time points in each of the months included in the study. Results are expressed as the mean  $\pm$  SEM for each group. Evaluation of the significance of differences between the means of parameters at different time points was performed using the One-way ANOVA. In all cases  $P \le 0.05$  was considered significant.

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decrease (p = 0.0001) being observed when compared to indoor afternoon as well as outdoor morning/afternoon counts; probably because of convection currents, as previously explained.

With respect to fungal diversity, based on macro- and micro-morphological characters, eight different isolates were recovered from the sampling site in this study. Six were identified through direct microscopic observation as *Peranospora parasitica*, *Phytophthora infestans*, *Dictyocha sterile*, *Geotricum candida*, *Aspergillus* spp. and *Penicillium* spp. Among these, highly aerobic and ubiquitous *Aspergillus* spp. were found to be dominant indoors as well as outdoors. Several studies on aeromycoflora in India reported their presence mainly in indoor areas such as libraries [25], hospitals, poultry farms, bakery, grain stocks, leather store houses, etc. Around 16 species of *Aspergillus* have been reported from a flour mill in India previously [26, 27]. *Aspergillus* spp. can cause a variety of pulmonary abnormalities including invasion of the lungs and their blood vessel leading to sepsis and death [28]. *Aspergillosis* is one of the commonest infections occurring in individuals with deficient immunity or as a secondary infection following inhalation of fungal spores [4].

Figure 4(A) shows the indoor and outdoor bio-aerosol concentrations within the temple premises. It can be observed that the indoor concentration throughout the study has always remained higher than the outdoor concentration. But, a statistically significant increase in the indoor concentration (p = 0.0001) in the month of July and September was noted. This may in general be attributed to poor ventilation inside the temple. Significantly higher indoor microbial load in July (peak monsoon season in India) was observed. An aerobiological study in Nigeria has also reported higher frequency of airborne bacterial isolates indoors in the rainy season than the dry season [29]. The increased indoor microbial concentration in September may additionally be caused by the large number of people visiting the temple as it is a festive season. Higher concentrations of airborne micro-organisms are usually found in indoor areas with greater public attendance and non-availability of airconditioners. Micro-organisms can be brought into the internal environment from outside



Figure 4. (A) Represents distribution of total microbial count indoor and outdoor in CFU/m<sup>3</sup> while (B) represents a comparative analysis of bacterial and fungal load in CFU/m<sup>3</sup> in each of the months included in the study. Results are expressed as the mean  $\pm$  SEM for each group. Evaluation of the significance of differences between the means of parameters at different time points was performed using the One-way ANOVA. In all cases  $P \le 0.05$  was considered significant.

Temperature	e R	Ή	Wind speed
-0.1000 0.5000	_	0.2000 0.6000	$0.2000 \\ -0.6000$
Table 4. ANOVA results	for distribution of total ba	acterial and fungal count.	
Distribution of l (refer Fig 2)	pacterial count	Distribution of (refer Fig 3)	fungal count
F value	P value	F value	P value
13 8 321	0.0004	1.347 0.8670	0.2977
14.67 2.533	0.0003 0.1062	36.23 5.797	0.0001 0.0109 0.0058
	-0.1000   0.5000   Table 4.   ANOVA results   Distribution of 1   (refer Fig 2)   F value   13   8.321   14.67   2.533	Table 4.ANOVA results for distribution of total baDistribution of bacterial count (refer Fig 2) $F$ value130.0004 8.3218.3210.0003 2.5330.1062	TemperatureKH-0.10000.20000.5000-0.6000Table 4. ANOVA results for distribution of total bacterial and fungal count.Distribution of bacterial countDistribution of crefer Fig 2)F valueP value130.00048.3210.00290.867036.232.5330.10625.797

Table 3. Spearman correlation coefficient 'r' showing the effect of meteorological factors on indoor and outdoor concentrations.

	F value	P value
Distribution of total microbial count (monthwise) (refer Fig 1)	2.905	0.0355
Distribution of total microbial count (indoor vs outdoor) (refer Fig 4A)	10	0.0001
Distribution of total microbial count (bacterial vs fungal) (refer Fig 4B)	2.626	0.0227

by visitors and their colonization on the surfaces of indoor objects can become an important source of contamination [30]. In addition to the number of occupants and ventilation, the microbial load in indoor air is also strongly influenced by various activities carried out in a closed environment [4]. Burning of incense, of oil lamps, etc. can result in excessive moisture exceeding the hydrocarbons limit, which leads to an 'out-of-balance' environment that tends to increase the indoor microbial pollution [31]. Likewise, September appears to have recorded a higher bacterial load as compared to fungi (Figure 4(B)). The difference in load has remained statistically insignificant throughout the study. The ANOVA results for the same have been summarized in Table 5.

Geography and climate play an important role in determining outdoor microbial concentrations. The most significant environmental factors influencing the viability of micro-organisms are temperature, RH and wind speed [4,32–35]. The levels of bacteria and fungi are strongly affected by these factors as they need specific environmental conditions to grow and propagate [36]. While temperature and water availability affect the source and particularly aid the release of fungal spores in the atmosphere, the wind speed helps in creating mechanical disturbance or strong air movement sufficient to disrupt materials and cause aerosolization [32].

In this study, outdoor microbial concentrations were found to correlate positively with temperature and indoor concentrations were negatively correlated; and in the case of RH and wind speed, indoor microbial concentrations positively correlated, but outdoor concentrations negatively correlated. Yet this correlation with the common meteorological parameters was found to be non-significant (Table 3). This could be explained by shorter sampling times. Our findings are consistent with reports from similar aerobiological studies in India carried out in other densely populated areas such as a railway station and public

garden at Gwalior and a vegetable market in Jabalpur where temperature, humidity and wind speed were not found to be any of the major factors responsible for aerosolization of micro-organisms [15,17].

#### Conclusion

This study reveals that indoor bio-aerosol concentrations are higher than outdoor indicating a potential problem for public health. Potential health effect such as hypersensitivity, respiratory, infectious diseases, acute toxic effects and allergies may arise from exposure of such large numbers of people to such high concentrations of bio-aerosols. Smaller cells and spores can easily get trapped within lung tissues [4] leading to severe respiratory disorders. On an average, a devotee spends more than 15–20 min in the temple during which time there is exposure to the microbial load in the air.

It is ideally recommended that heating, ventilation and air conditioning systems be designed indoors to prevent the entry of bio-aerosols and to maintain conditions within a closed environment that do not help microbial growth. This may be impossible within places such as temples where alternative interventions such as electronic air cleaners or simpler exhaust fan systems may be used. Indoor areas with air-conditioners and/or cooling systems need to have a maintenance schedule so as to remove dirt from their internal components as well as from fan coil units. Factors to be taken into consideration while designing enclosed places, in order to avoid high microbial load, are appropriate air intake and good air filtration. High Efficiency Particulate Air filters or activated carbon and microbiocide-treated filters can also be used wherever possible thus reducing the microbial air load and increasing the health hygiene in temple environments.

#### Abbreviations

NEERI – National Environmental Engineering Research Institute; PBS – Phosphate Buffered Saline; CFU – Colony Forming Units; HVAC – Heating Ventilating Air Conditioning; HEPA – High Efficiency Particulate Air; ANOVA – Analysis of Variance; SEM – Standard Error Mean.

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