

Influence of seasonal variation on concentration of fungal bioaerosol at a sewage treatment plant (STP) in Delhi

Sunita Maharia · Arun Srivastava

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Abstract Ambient levels of viable bioaerosol (fungi) were measured at six different sections of a sewage treatment plant in Vasant Kunj, New Delhi. The sampling was carried out for fungal fractions of bioaerosols during the month of June 2012 to May 2013 covering all the four seasons of India, i.e., monsoon, post-monsoon, winter, and pre-monsoon. Sampling was done thrice in a month with the help of a six-stage viable cascade impactor for 10 min each at a flow rate of 28.3 l min^{-1} . Temperature and relative humidity were also recorded during each sampling. It was found that among all the six sites, maximum and minimum fungal concentrations were $1,200$ and 829 CFU m^{-3} in pre-monsoon; $1,198$ and 802 CFU m^{-3} in monsoon; $1,289$ and 868 CFU m^{-3} in post-monsoon; and 516 and 279 CFU m^{-3} in winter seasons, respectively. A good regression of fine over coarse fungal bioaerosols was observed in all the four seasons. In total, eight genera of fungi were identified, and among the eight genera identified, four, e.g., *Mucor*, *Rhizopus*, *Aspergillus*, and *Penicillium*, were found in maximum number during all the seasons. This study has revealed interesting results in context to the relationship between concentrations of

fungal bioaerosols and environmental factors as well as interparticle size relationship of fungal bioaerosols.

Keywords Bioaerosol · Fungi · Sewage treatment plant · Delhi

1 Introduction

The rapid urbanization in recent years has led to the construction of a large number of sewage treatment plants (STPs), compost plants, and landfill sites in India. Such plants accumulate solid wastes, which is a significant source of bioaerosol emission. STPs are recognized as being an important source of generation of microbial aerosols and may constitute a health hazard for plant workers and the surrounding population (Orisini et al. 2002; Fracchia et al. 2006; Grisoli et al. 2009). Microbiological pollution emitted from wastewater treatment plants (WWTP) contains *Actinomyces* (*Mycobacterium tuberculosis*), viruses (Hepatitis A virus, polio viruses, coxsackie viruses, echo viruses, rota viruses, adeno viruses, Norwalk virus), bacteria (*Salmonella* spp., *Shigella* spp., *Campylobacter jejuni*, *Yersinia enterocolitica*, *Legionella pneumophila*, *Helicobacter pylori*, *Listeria monocytogenes*, *Mycobacterium xenopi*), parasitic protozoa (*Giardia lamblia*, *Entamoeba histolytica*), and helminths (De Luca et al. 1998; Sneath et al. 1986). Thus, STP workers and people living in the vicinity are

S. Maharia · A. Srivastava (✉)
School of Environmental Sciences, Jawaharlal Nehru
University, New Delhi 110 067, India
e-mail: srivastava02@hotmail.com;
a_srivastava@mail.jnu.ac.in

prone to exposure to pathogens and endotoxins, most likely through the inhalation of aerosols or by the hand-to-mouth route.

The sewage and sewage sludge are the major sources of bioaerosol, where pathogenic microorganisms are found. In these sites, microorganisms find suitable conditions for their growth or survival; hence, their survival time becomes longer ranging from some days to several dozen days (Brandi et al. 2000; Taha et al. 2005). Each of the sources contains a vast number and variety of microorganisms including pathogens. The concentration of airborne microorganism varies widely between different studies due to the differences in treatment processes of wastewater and sludge in climatic condition as well as in sampling and analysis techniques (Bausum et al. 1982; Pillai et al. 1996; Brenner et al. 1988).

Number of studies have been conducted, which resulted potential health risk to workers exposed to aerosols from sewage (Clark et al. 1976; Ferris et al. 1999; Rylander 1999; Thorn and Kerekes 2001; Hansen et al. 2003; Jeggli et al. 2004; Rylander et al. 1976). The continuous exposure to large concentration may lead to the development of occupational disease such as allergic alveolitis, asthma and organic dust toxic syndrome, and sewage worker syndrome (Fannin et al. 1985; Lacey and Crook 1988; Lacey and Dutkiewicz 1994; Clark 1987; Swan et al. 2002).

Subsequently, the purpose of this work was to collect a comprehensive dataset for fungal concentration in different size ranges in different seasons and at different sites of a STP, an outdoor environment. The reason behind choosing such a sampling site is that the workers at wastewater treatment plants are exposed to pathogenic organisms through the air they breathe and they also spend more time in areas where the potential for higher exposure exists. The site where experiment has been conducted is one of the STPs with potential of significant microbial contamination and thus poses the great health risks particularly to the plant workers as well as adjoining human population and animals.

2 Materials and methods

2.1 Description of the study area

Bioaerosol sampling was carried out at a STP in New Delhi, which lies in the latitude 28° 25' and longitude

of 76° 50'. Delhi is the capital city of India and situated in the Northern part of the country. It has a population density of 29,155 persons/Km². Its population has rapidly increased from 3.5 million in 1970 to over 22 million in 2011 (Census of India 2011). The climate of Delhi is humid subtropical with hot summers and moderately cold winters. The monthly mean temperature range varies between 14.3 °C in January the coldest month, to about 34.5 °C in June the hottest month.

2.2 Sampling sites

Sampling was carried out at six different sections of STP situated in Vasant Kunj that is in the southwest part of Delhi. This STP lies in the close vicinity of Jawaharlal Nehru University (JNU), New Delhi. The STP was commissioned in 1985 by the Delhi Development Authority (DDA) and was later passed by the Delhi Jal Board (DJB) on December 19, 1988. DJB is responsible for collection, transportation, and treatment of sewage in Delhi. The six sampling sites were selected on the basis of on-site survey. Their characteristic details are given below and illustrated in Fig. 1.

2.2.1 Inlet 1

The site Inlet 1 is situated on the western part of STP. It is the entry point of the raw sewage into the treatment plant. At this inlet, two separate pipe lines of capacity 4 MLD (million liters per day) and 3 MLD carry sewages from two regions namely Kishangarh and Vasant Kunj area. The surrounding area of Inlet 1 is covered with green vegetation.

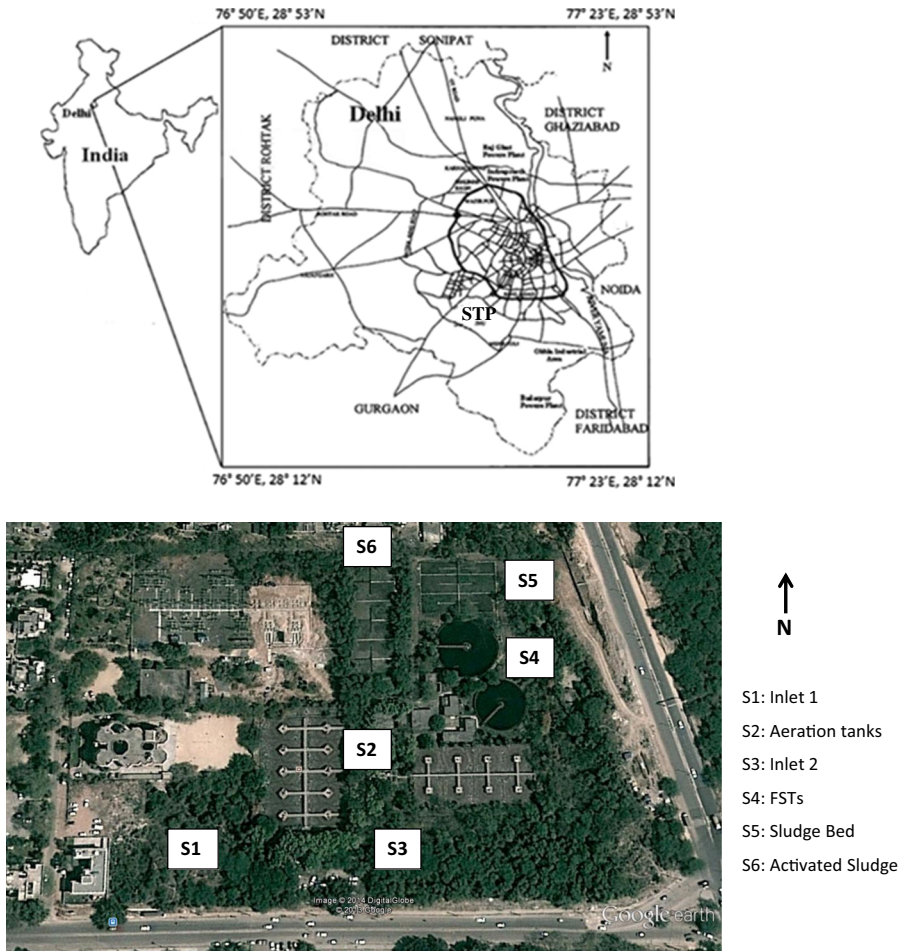
2.2.2 Inlet 2

Inlet 2 is situated on the eastern part of STP. It carries approximately 15 MLD of sewage from Vasant Kunj area. The opening of Inlet 2 consists of both bar screen and grit chamber to remove huge waste products such as cans, plastic bags, cloths, and also fine debris from the flowing sewage.

2.2.3 Aeration tank

Aeration Chamber is situated in the middle part of STP. Its capacity is around 15 MLD. Sewage from two

Fig. 1 Study map of sewage treatment plant, Vasant Kunj (New Delhi)



inlets (1 and 2) is collected in this Aeration Chamber. In the Aeration Chamber, aerobic treatment is used to promote oxidation and microbial decomposition of organic matter by bacteria through biological process.

2.2.4 Final settlement tank (FST)

There are two FSTs. They are circular chambers of around 30–35 m diameter and 6 feet deep. The water from the Aeration Chamber then moves into the settling tank. Here, in the settling tank, the sewage flow slows down due to gravity, the flocculent sludge settles down on the bottom of the FST.

2.2.5 Activated sludge

Activated sludge is an open chamber of 60 × 44 m dimension. Here, air and oxygen are introduced into

the mixture of screened primary treated sewage and wastewater combined with microorganism to develop a biological flocculent sludge that reduces the organic content of sewage.

2.2.6 Sludge bed

Sludge bed is a bed of rectangular shape. There were two sludge beds of dimensions 27 × 64 and 31 × 45 m. In these sludge beds, final treated sludge is stored. Afterward, dewatering and disposal of solid and liquid waste collected from the settling tanks take place. Sludge, thus formed, is disposed off to agricultural and vegetation fields where it is used as organic manure.

2.3 Bioaerosol sampling

Bioaerosol sampling for fungi was done during June 2012 to May 2013 thrice in each month at all the six

Table 1 Jet orifice diameter and particle size range for each stage

Stage	Orifice diameter (mm)	Size range (μm)
1	1.18	>7.0
2	0.914	7.0–4.7
3	0.711	4.7–3.3
4	0.533	3.3–2.1
5	0.343	2.1–1.1
6	0.254	<1.1

STP sections. These 12 months (June through May) cover more or less all the four different seasons of India, i.e., pre-monsoon, monsoon, post-monsoon, and winter season. Therefore, a total of 18 samples in each month or 54 sets (with 6 samples per set) of samples in each season (one season covers three months) were obtained.

The sampler used was a six-stage viable cascade impactor (TE-10-800), manufactured by Tisch Environmental Inc., USA. Cascade impactor consists of a stack of six impaction stages. The details of the size range of the particles and their orifice diameter are provided in Table 1. Cascade impactor is normally used to measure the particle size distribution and concentration of aerobic bacteria and fungi in ambient air. Various stages were consisting of one or more nozzles and a target or substrate. The sampling was carried out for 10 min at a flow rate of 28.3 LPM at an average height of 1 m above ground level.

For the detection and enumeration of fungi, PDA was used as media. PDA is a common microbiological media made from potato infusion and dextrose (corn sugar). Potato infusion and carbohydrate promote the growth of fungi while the presence of low pH and antibiotic inhibits the growth of bacteria. Fungal fraction of bioaerosol was collected over potato dextrose agar media (ACGIH 1999; Baron and Finegold 1990; Smith 1990).

2.4 Meteorological parameters monitoring

Considering the strong dependence of survival and growth of bioaerosols on the meteorological conditions, the monitoring of various meteorological parameters was carried out. Meteorological parameters such as

temperature, relative humidity (RH), and wind velocity were recorded using Envirometer (Fisher Scientific).

2.5 Bioaerosol characterization

2.5.1 Sample preparation

While performing the experiment, inoculated agar plates were incubated for 4 days at 25 °C to develop a visible colony.

2.5.2 Bioaerosol estimation

The concentration (in units of CFU/m³) of culturable microorganisms is calculated by dividing the volume of air sampled from the total number of colonies observed on the plate. A colony is a macroscopically visible growth of microorganisms on a solid culture medium. Concentrations of culturable bioaerosol normally are reported as colony-forming units (CFU) per unit volume of air sampled. CFU is the number of microorganisms that can replicate to form colonies, as determined by the number of colonies that develop.

Bioaerosol concentration (CFU/m³)

$$= \frac{\text{No. of colonies}}{\text{Flow rate} \times \text{Sampling duration (min)}}$$

$$= \frac{\text{No. of colonies}}{0.0283 \times \text{Sampling duration (min)}}$$

where flow rate = 28.3 l/min = 0.0283 m³/min.

2.5.3 Identification

A small portion of fungal colony is taken with the help of inoculums loop and placed on to a slide containing 4 % of NaCl. A drop of lactophenol cotton blue stain is added over it immediately and left for about 1–2 min. The area is then covered by a coverslip, and it gets ready for microscopic examination and visual identification.

2.5.4 Data analysis

All the statistical analyses including regression and correlation were done using Microsoft Excel 2010.

3 Results and discussion

3.1 Size-segregated distribution of various fractions of fungal bioaerosol

The concentration of fungal bioaerosol found in different stages at each site seems to follow a typical pattern in all the four seasons. As shown in Figs. 2, 3, 4, 5, there is an increasing trend in the concentration of fungal bioaerosol from stage 1 to stage 4 with a further decrease from stage 4 to stage 6. In all the four figures, the highest concentration of fungus is found in stage 4 (diameter ranging from 2.1 to 3.3 μm) and lowest concentration in stage 6 (diameter ranging from 0.6 to 1.1 μm). The distinctive pattern of fungal concentration shows that majority of the fungal species have similar diameter as that of stage 4, which corresponds to the secondary bronchi of the lungs in human body. These data reveal that the majority of the immune toxic and allergic fungi found at stage 4 are mostly prone to affect the secondary bronchi in human lungs when inhaled.

3.2 Seasonal variation in fungal bioaerosol concentration

The seasonal variation in fungal concentration is provided in Fig. 6, and the temperature and RH at each site are shown in Table 2. Among all the six sites, maximum and minimum fungal concentrations were 1,289 and 868 CFU m^{-3} at a temperature range of 24–25.4 $^{\circ}\text{C}$, respectively, in post-monsoon season. In monsoon, low fungal concentration range (802–1,198 CFU m^{-3}) was found at temperature range of 32–34 $^{\circ}\text{C}$ in all sections of STP. Around 829–1,200 CFU m^{-3} of

fungal concentration was found at temperature range of 30–31.3 $^{\circ}\text{C}$ during pre-monsoon season. In winter season, very less fungal concentration range (279–516 CFU m^{-3}) was found because of low temperature and low humidity.

In STP, the fungal concentrations in samples were less, compared to some other studies (Korzeniewska et al. 2009; Pietro et al. 2008; Lin et al. 2011; Zofia et al. 2003). Korzeniewska et al. (2009) reported a mould concentration ranging from 1,000 to 5,000 CFU/m^3 at different sites of WWTP. Pietro et al. (2008) reported microfungal concentration ranging from 162 to 4,313 CFU/m^3 . Lin et al. (2011) found the fungal concentration ranging from 141 to 1,590. In STP (Poland), the minimum fungal concentration range recovered was 24–140 CFU/m^3 (Zofia et al. 2003). There are some bioaerosol studies that have been conducted whose results are compared to the present study (Ghosh et al. 2013; Fang et al. 2005). The differences in fungal concentration in different studies could be explained by different sampling sites and sampling methods used to measure fungal concentrations, air flow rate, duration of sampling, and culture medium.

Detailed study of bioaerosol distribution in different seasons revealed a pattern in which fungi is found to be at their maximum concentration in post-monsoon season in comparison with monsoon, pre-monsoon, and winter period. In monsoon season, even though temperature and RH were found to be at optimum range for fungal bioaerosol growth yet, rain washout is the major reason of less fungal concentration in comparison with post-monsoon season. In case of pre-monsoon season, high temperature and correspondingly low RH justify the reason for less fungal

Fig. 2 Total fungal concentration at each stage of the sampler in monsoon period

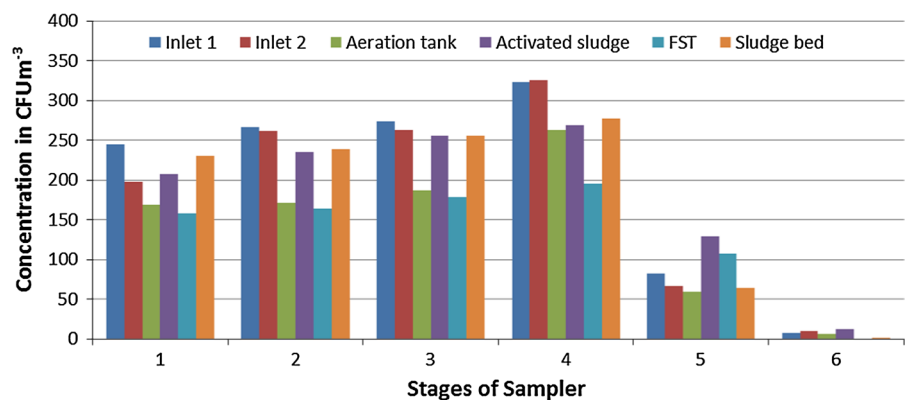


Fig. 3 Total fungal concentration at each stage of the sampler in post-monsoon period

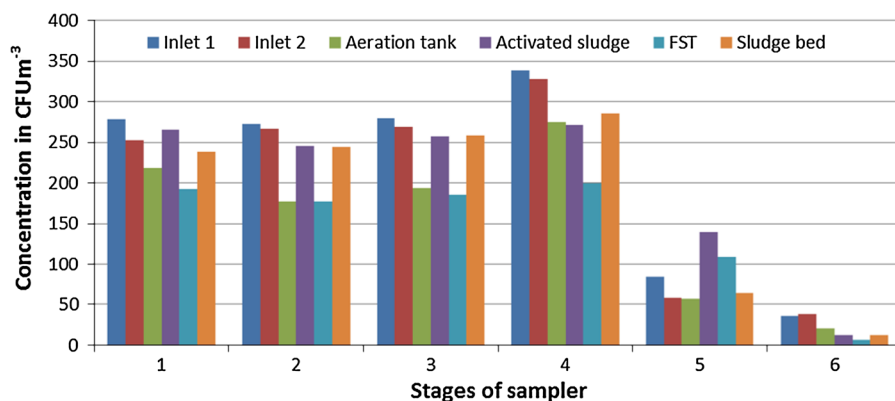


Fig. 4 Total fungus concentration at each stage of the sampler in winter period

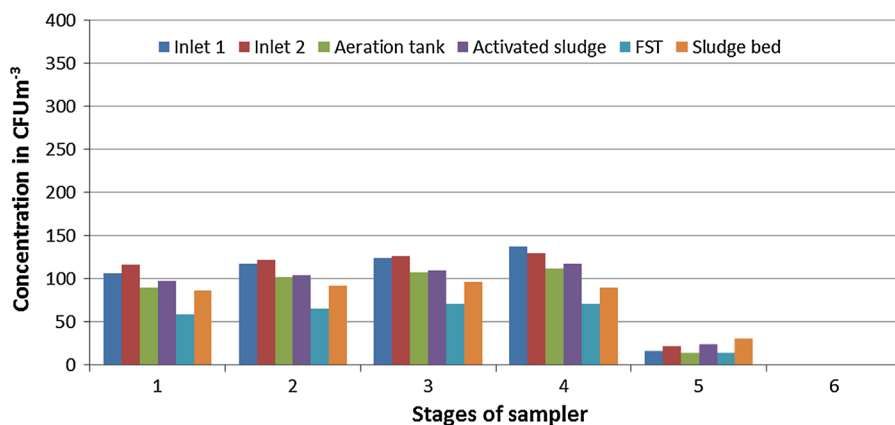
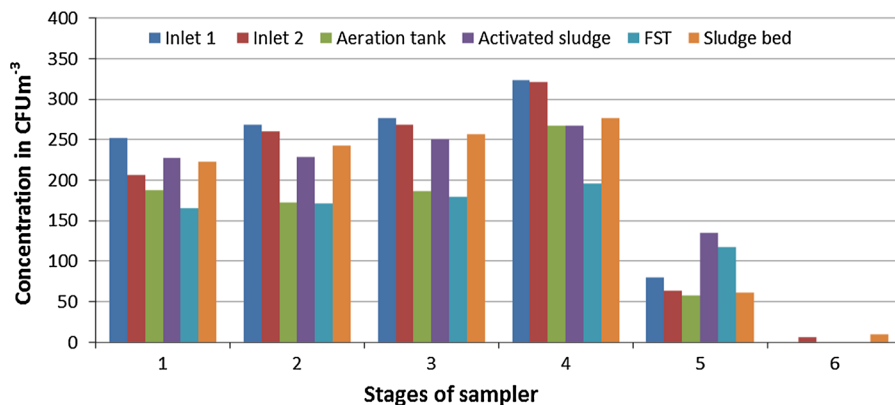


Fig. 5 Total fungus concentration at each stage of the sampler in pre-monsoon period



concentration while relatively optimum temperature and high RH are the reasons for elevated fungal concentration in winter season.

3.3 Regression analyses

Regression analyses were carried out between coarse and fine fractions of fungal bioaerosols. The sum of

stages 1–3 was considered as coarse fraction while sum of stages 4–6 was considered as fine fraction. Various sites were presumed as the repetition of data. Coarse mode bioaerosols were considered as dependent while fine as independent variable. Results for different seasons are shown in Figs. 7, 8, 9, 10. It is evident from these figures that a good regression in monsoon ($R^2 = 0.855$), winter ($R^2 = 0.728$), and pre-

monsoon ($R^2 = 0.751$) seasons suggests that the variation in coarse fractions of bioaerosols is largely explained by the variance in fine fractions of bioaerosols. However, a less significant value of R^2 (0.525) in post-monsoon season implies that the variations in coarse mode bioaerosols were less correlated. The concentrations of coarse and fine bioaerosols might be determined by different environmental factors.

3.4 Correlation analysis

Correlation analyses of bioaerosol concentration were carried out between the different stages as well as of each stage with total. Good correlation existed between different fractions of fungal bioaerosol with each other and with total. The only exception is stage 6, which is not at all correlated with any of the stages. As shown in Table 3, very good correlation exists among the different stages in monsoon season, such as stage 1 with stage 2 ($r = 0.8663$); stage 1 with stage 3 ($r = 0.9183$); stage 1 with stage 4 ($r = 0.8514$); stage

2 with stage 3 ($r = 0.8895$); and stage 3 with stage 4 ($r = 0.8780$). Therefore, it can be concluded that stage 1 has maximum number of correlation followed by stage 2, stage 3, and stage 4 while stage 5 and stage 6 do not have any correlation with any of the stages. The reason behind such correlation result is due to the fact that most fungal spores remain attached with different sizes of soil and other particulate matter, thus remain segregated according to their size in different stages of the sampler. Apart from this, it is also a fact that the size of most fungal spores exists in the range of stages 1–5, and very low concentrations were observed in stage 6.

Again, in post-monsoon season, good correlations exist among stages 1–4 with the total concentration and stage 1 with stage 2, and average correlation exists among different stages such as stage 1 with stage 3, stage 1 with stage 4. In winter season, there was no correlation found between stage 6 and other stages while good correlation was found between stages 2

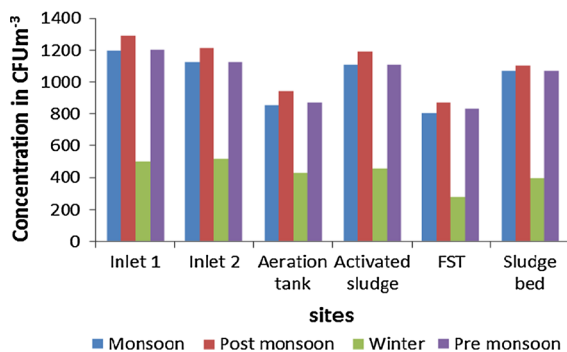


Fig. 6 Comparison of total fungal concentration at different sites in different season

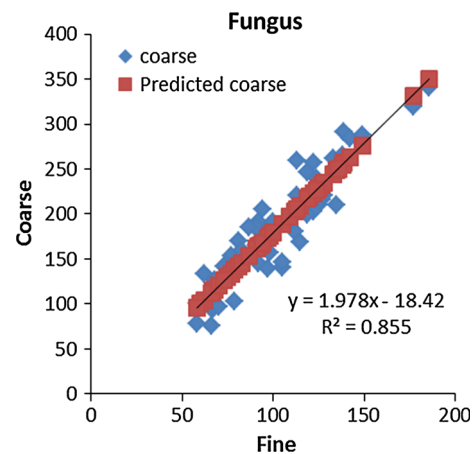


Fig. 7 Plot between coarse and fine fungal concentration in monsoon season

Table 2 Average temperature and relative humidity (RH) during each sampling

Sites	Inlet 1		Inlet 2		Aeration tank		Activated sludge		FST		Sludge bed	
	Temp (°C)	RH (%)	Temp (°C)	RH (%)	Temp (°C)	RH (%)	Temp (°C)	RH (%)	Temp (°C)	RH (%)	Temp (°C)	RH (%)
Pre-monsoon	31.3	44.9	31	44.8	31	45	30.5	44.5	30	46	31	45.6
Monsoon	34	57.1	33.5	58	32	59.3	32.5	58	32	59	31.5	59.5
Post-monsoon	25	66.4	24	67.3	25	67	25.5	68	24.5	66.5	25	66
Winter	15.1	77.6	13.11	76	14	78.3	14.5	77.8	13	76.5	13.5	75

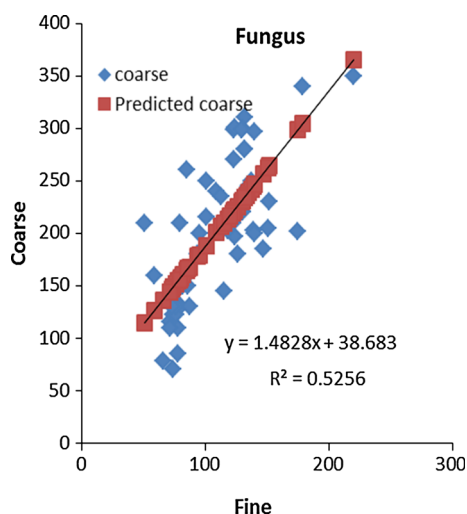


Fig. 8 Plot between coarse and fine fungal concentration in post-monsoon

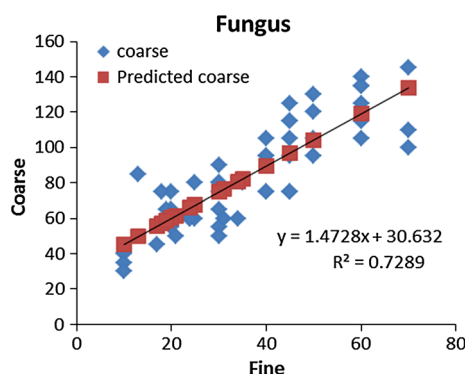


Fig. 9 Plot between coarse and fine fungal concentration in winter season

and 4, stages 3 and 4, and stages 1–4 with total concentration. It is also evident that in case of fungi, in pre-monsoon season, good correlation was found between stages 1 and 3 ($r = 0.7880$), stages 1 and 4 ($r = 0.7405$), stages 3 and 4 ($r = 0.8445$), and very good correlation exists between stages 1–4 with the total concentration.

3.5 Identification of fungal bioaerosol

Eight genera of fungal were identified in bioaerosols at all the six sections of STP during four different seasons as shown in Table 4. Here, it is pertinent to mention that we presumed that the distribution

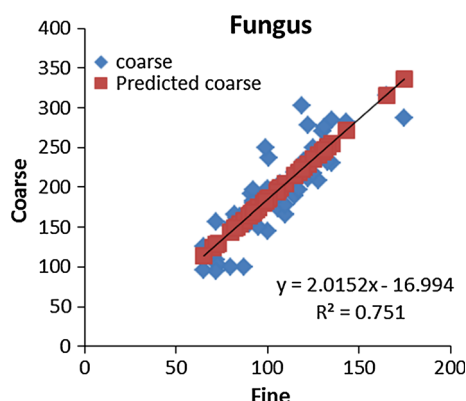


Fig. 10 Plot between coarse and fine fungal concentration in pre-monsoon season

through the air stream of fungi on petri plates is uniform. Also, since we did repeated sampling and picked up randomly a small portion of fungal colony, it will not be biased for some particular genus or genera. Eight genera were identified, and four genera that were found in maximum number in all the seasons were *Mucor*, *Rhizopus*, *Aspergillus*, and *Penicillium*. Concerning the number of fungal genera isolated from different section of STP during the study period (Table 4), it was noticed that many of the fungal genera that recovered were similar to those observed in some other studies. The *Penicillium* and *Aspergillus* were always the most frequently identified genus, which supported the other results (Sautour et al. 2009). Ren et al. (1999) showed that *Aspergillus*, *Penicillium*, *Cladosporium*, and *Alternaria* are the predominant genera in outdoor air (Table 4). Ebner et al. (1989) found species of *Aspergillus* in high concentrations especially in late fall and following rainy weather. Ghosh et al. (2013) conducted a bioaerosol study in indoor environments of Delhi and found *Aspergillus* as a most prominent fungus.

The species of *Penicillium* have been identified as important causative agents of extrinsic bronchial asthma (Shen and Han 1998). It has been reported that most common genera namely *Aspergillus*, *Penicillium*, *Cladosporium*, and *Alternaria* should always be considered as a cause of fungal allergy (Peat et al. 1993). In the present study, although some degrees of seasonal variations in the major genera were detected, the most notable ones were the *Aspergillus* and *Penicillium*. The *Penicillium* is harmful for sewage

Table 3 Correlation matrix between different size fractions for fungi in various seasons

Season	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6	Total
<i>Pre-monsoon</i>							
Stage 1	1						
Stage 2	0.6736	1					
Stage 3	0.7880	0.7005	1				
Stage 4	0.7405	0.6537	0.8445	1			
Stage 5	0.1821	0.0206	0.1350	−0.0916	1		
Stage 6	0.3549	0.3195	0.2859	0.2116	−0.0471	1	
Total	0.8963	0.8354	0.9364	0.8826	0.1859	0.3416	1
<i>Monsoon</i>							
Stage 1	1						
Stage 2	0.8663	1					
Stage 3	0.9183	0.8895	1				
Stage 4	0.8514	0.8730	0.8780	1			
Stage 5	0.3691	0.1980	0.3067	0.1277	1		
Stage 6	0.3870	0.4046	0.5354	0.4436	0.2193	1	
Total	0.9542	0.9410	0.9686	0.9311	0.3538	0.4988	1
<i>Post-monsoon</i>							
Stage 1	1						
Stage 2	0.6713	1					
Stage 3	0.5327	0.5705	1				
Stage 4	0.6437	0.6311	0.5945	1			
Stage 5	0.1947	0.1350	0.1677	0.1047	1		
Stage 6	0.2091	0.3031	0.1801	0.3563	−0.3603	1	
Total	0.8427	0.8451	0.8002	0.8527	0.2750	0.2998	1
<i>Winter</i>							
Stage 1	1						
Stage 2	0.6736	1					
Stage 3	0.7880	0.7005	1				
Stage 4	0.7405	0.6537	0.8445	1			
Stage 5	0.1821	0.0206	0.1350	−0.0916	1		
Stage 6	0.3549	0.3195	0.2859	0.2116	−0.0471	1	
Total	0.8963	0.8354	0.9364	0.8826	0.1859	0.3416	1

Bold digits: good correlation ($R > 0.500$) at significance level of $p < 0.05$ (i.e., at 95 %)

worker (Shen and Han 1998) while *Aspergillus* is allergic (with potential to produce carcinogenic Ochratoxin A and Aflatoxins), and *Rhizopus* is immunotoxic in nature. *Fusarium* and *Alternaria* were found in abundance in post-monsoon and pre-monsoon season, respectively. In monsoon period, major concentration of *Dreschelia*, *Mucor*, *Penicillium*, and *Aspergillus* was

found in each section of STP. The major concentration of *Fusarium*, *Rhizopus*, *Aspergillus*, and *Penicillium* was found at each section of STP in post-monsoon period. The important fungal genera (*Alternaria*) were found in high concentration in each site of STP during pre-monsoon period. *Alternaria* was absent in winter season.

Table 4 Fungal genus characterized at different sites of sewage treatment plant

Section	Species	Asperigillus ②	Fusarium ③	Drechslera ②	Ulocladium ①	Pencillium ①	Alternaria ③	Mucor ①	Rhizopus ③
Inlet 1	Pre-M	+			+	+	+	+	+
	M	+		+		+	+	+	+
	Post-M	+	+			+	+	+	+
	W	+				+		+	+
Inlet 2	Pre-M	+			+	+	+	+	+
	M	+		+		+	+	+	+
	Post-M	+	+			+	+	+	+
	W	+				+		+	+
Aeration tank	Pre-M	+			+	+	+	+	+
	M	+		+		+	+	+	+
	Post-M	+	+			+	+	+	+
	W	+				+		+	+
Activated sludge	Pre-M	+			+	+	+	+	+
	M	+		+		+	+	+	+
	Post-M	+	+			+	+	+	+
	W	+				+		+	+
FST	Pre-M	+			+	+	+	+	+
	M	+		+		+	+	+	+
	Post-M	+	+			+	+	+	+
	W	+				+		+	+
Sludge bed	Pre-M	+			+	+	+	+	+
	M	+		+		+	+	+	+
	Post-M	+	+			+	+	+	+
	W	+				+		+	+

① = Harmful; ② = Allergic; ③ = Immunotoxic

+, Present; Pre-M, Pre-monsoon; M, Monsoon; Post-M, Post-monsoon; W, Winter

4 Conclusions

From this study, it can be concluded that the concentration of fungal bioaerosol was found maximum in post-monsoon due to optimum temperature and RH and minimum in winter season due to low temperature. In all the four seasons, highest fungal concentration was found in stage 4 and lowest concentration at stage 6. As stage 4 has a diameter range of 2.1–3.3 μm (synonym to the secondary bronchi of the lungs in human body) hence, the presence of the majority of the immunotoxic and allergic fungi at stage 4 reveals when inhaled secondary bronchi of the STP workers are mostly affected.

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