

Temporal Variations in Fungal Bioaerosols in Outdoor Environment: A Three Year Study at Four Locations in Gwalior, Central India

Pramod Kumar and Ajay K. Goel*

DRDO-Defence Research & Development Establishment, Jhansi Road, Gwalior-474 002, India

*E-mail: akgoel73@yahoo.co.uk

ABSTRACT

Airborne fungi may cause a variety of health problems in human and animals. In this study, a survey of fungal bioaerosols was done for three years during 2007-2009 in Central India. Air samples were collected from four different locations at Gwalior, Central India on monthly basis. Results showed that fungal bioaerosols concentration ranged from 550 to 7363 CFU/m³ at different sites. Significant higher bioaerosols content was observed during the year 2008 and 2009 than 2007. Highest mean fungal concentration (2687.86 CFU/m³) was found at a public garden, whereas lowest mean concentration (1722.72 CFU/m³) was observed at civil hospital. A seasonal rhythm was observed in the level of airborne fungi. Maximal fungal count was observed in winter followed by monsoon and lowest in summers. Among meteorological factors, statistically significant negative correlation was found with temperature and wind speed. During the study, a total of 41 fungal species belonging to 21 genera were identified. *Alternaria*, *Aspergillus*, *Cladosporium*, *Curvularia*, *Fusarium*, *Penicillium*, *Phoma* and *Trichothecium* were the dominated genera. Several identified fungal species viz. *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus niger*, *Penicillium citrinum*, *Penicillium chrysogenum*, *Fusarium moniliforme*, *Trichoderma harzianum* and *Rhizopus stolonifer* from the studied area are well known for causing allergy or production of mycotoxin.

Keywords: Airborne fungi; Temperature; Humidity; Wind speed; Seasonal distribution

1. INTRODUCTION

Airborne fungi are among the most abundant organisms in nature. In last few years, aeromycological research has gained impetus due to awareness of health consequences associated with airborne fungi. Airborne fungi can directly or indirectly affect human, animal, plant and environment¹. Direct human health effects are associated with allergic reactions, eye and respiratory irritations, skin infection and toxicity². Indirect effects include spoilage of food stuffs, biodeterioration of archival and building materials³. Elevated microbial load is associated with increased probability of health problems like decreased lung function, increased respiratory symptoms and asthma attacks, cardiovascular disease and lung cancer^{4,5}.

Generally, fungi enter into atmosphere from vegetation and soil due to anthropogenic activities, traffic and natural process. Once they become aerosolised, their survival and distribution is modulated by various meteorological and climatic conditions⁶. Fungal concentration varies according to geographical locations, seasonal variations and anthropogenic activities⁷⁻⁹. Environmental conditions such as relative humidity, temperature and wind velocity exert significant effects on the type of population and amount of microorganisms in the air^{6,10}. We have observed variations in fungal bioaerosols with two different locations in Gwalior associated with week

days activities¹¹. We have also reported distribution pattern of fungal bioaerosols for a short time over Gwalior-Fair ground associated with a large public gathering¹². Present study was conducted at Gwalior, Central India to find out the species, number and distribution of airborne fungi at different locations in outdoor environment over a period of three year.

2. MATERIALS AND METHODS

2.1 Air Sampling

Air sampling was done at four different locations during 2007-2009 on monthly basis in Gwalior, a historical city in Central India (longitude 78° 13' E, latitude 26° 13' N). The weather of Gwalior can be broadly divided into three season: summer (March to June), rainy season (July to October), and winter (November to February) based on temperature, relative humidity, and rainfall levels. The sampling sites were railway station circle (RLY), Dal bazaar market (MAR), Jai Arogya Hospital (JAH) and a public garden (GAR). The first location, railway station circle was selected because here traffic remains fairly steady throughout the day. Traffic consisted primarily of auto rickshaws, cars, two wheelers and foot traffic. The market site was selected as this is densely populated and also associated with traffic. The third sampling location was a public garden, located in the city which remained mostly free of automobile traffic. The hospital site was selected for the public awareness to control fungi mediated nosocomial infections.

Table 1. Meteorological parameters temperature, wind speed and relative humidity during 2007-2009

		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	
Temperature (°C)	2007	RLY	20	25	34	37	38	39	32	33	34	31	30	24
		MAR	21	25	34	38	40	40	34	34	34	31	31	26
		JAH	21	27	35	39	40	42	33	35	35	32	31	25
		GAR	21	26	32	33	40	40	26	32	30	31	30	24
	2008	RLY	19	21	32	39	40	35	34	29	27	35	23	26
		MAR	19	22	33	39	41	36	34	31	27	36	25	28
		JAH	20	22	33	40	41	37	35	31	26	37	26	29
		GAR	19	22	30	38	39	36	30	30	25	35	23	26
	2009	RLY	21	24	32	40	41	44	34	33	34	32	25	24
		MAR	22	24	36	41	42	45	36	33	37	32	26	25
		JAH	22	25	34	41	45	43	35	34	36	33	27	28
		GAR	20	24	35	40	41	42	32	31	33	32	24	24
Wind speed (m/s)	2007	RLY	0.4	0.6	0.7	2.3	1.8	1.5	1.3	0.8	1.4	1.6	0.8	0.7
		MAR	0.4	0.8	0.9	2.1	2.7	1.1	2	0.7	2.1	1.5	1	0.6
		JAH	0.2	0.5	0.4	1.4	1.3	0.9	0.7	0.9	1.2	0.5	0.4	0.7
		GAR	0	0.2	0.4	0.8	1	0.7	0.4	0.6	0.7	0.5	0.3	0.3
	2008	RLY	0.6	0.4	0.7	1.3	1.5	1.2	0.4	0.6	0.6	0.5	0.6	0.3
		MAR	0.4	0.5	0.9	0.8	1.9	1	0.5	0.5	0.4	0.4	0.3	0.5
		JAH	0.5	0.2	0.6	0.5	1.3	0.7	0.3	0.3	0.4	0.2	0.2	0.3
		GAR	0.3	0.2	0.4	0.6	1.1	0.8	0.2	0.4	0.2	0.2	0.3	0.4
	2009	RLY	0.7	0.4	0.7	1	0.8	1.4	0.5	0.6	0.7	0.6	0.4	0.3
		MAR	0.5	0.3	0.5	0.8	1.2	1	0.3	0.8	0.5	0.4	0.2	0.3
		JAH	0.3	0.2	0.3	0.5	0.7	0.8	0.1	0.4	0.9	0.3	0.2	0.2
		GAR	0.3	0.2	0.2	0.3	0.5	0.6	0.2	0.3	0.5	0.4	0.1	0.2
Humidity (%)	2007	RLY	31	29	27	19	24	25	41	38	42	26	24	24
		MAR	30	29	26	18	22	23	44	36	35	24	23	25
		JAH	32	27	24	18	21	24	40	39	34	22	23	28
		GAR	35	30	26	19	29	28	42	43	40	29	25	33
	2008	RLY	27	24	24	17	29	34	53	47	72	30	25	30
		MAR	25	25	26	16	27	31	55	46	71	22	24	24
		JAH	25	25	26	16	27	31	55	46	71	22	24	24
		GAR	29	26	27	21	28	36	70	55	79	32	28	29
	2009	RLY	30	27	25	18	21	15	52	46	54	36	34	32
		MAR	31	26	25	17	18	15	55	40	43	35	33	30
		JAH	27	25	29	17	19	16	42	39	41	33	29	26
		GAR	29	28	30	20	22	19	58	49	66	35	34	30

2.2 Sampling Procedure

The sampling procedure was adopted from our earlier report. In brief, air samples for fungal bioaerosols analyses were collected using Router Centrifugal Sampler (Biotest, Germany) on Rose Bengal Agar (Difco Laboratories, Detroit, MI) at a height of 1.5 m from the surface to simulate human breathing zone. Fungi were identified based on their culture characteristics, sporulation pattern and microscopic features¹¹.

2.3 Meteorological Data

Temperature, RH and wind speed were recorded at each location with handheld Envirometer (Fisher Scientific, Control Company, TX, USA). The meteorological data for three year has been given in Table 1.

2.4 Statistical Analysis

Fungal load at different sites were compared using one way analysis of variance (SigmaPlot 2000). Year wise and seasonal comparisons were made according to student t-test.

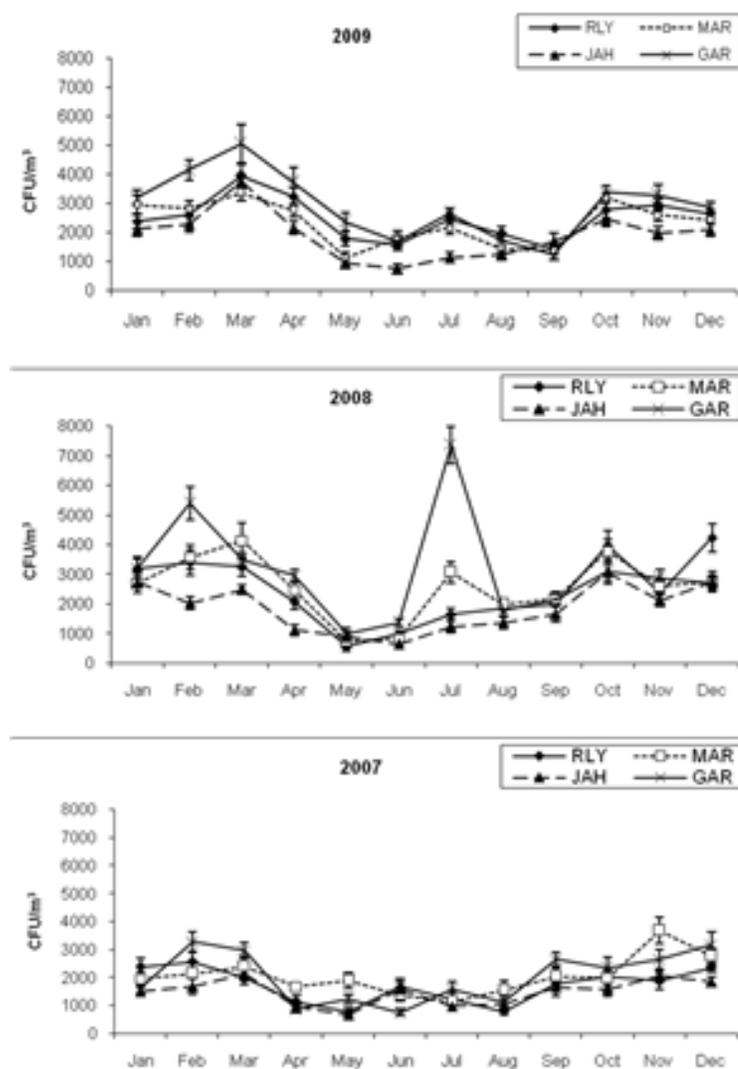


Figure 1. Total fungal counts at four different locations at Gwalior during 2007-2009.

Relationship between fungal counts and meteorological factors was examined by Spearman correlation analysis. A probability of less or equal to $P \leq 0.05$ was considered significant.

3. RESULTS

3.1 Concentration of Fungal Bioaerosols

The count of fungal bioaerosols at different sites varied from 550 to 7363 CFU/m³. The mean concentrations observed were 1810.17±707.9 CFU/m³, 2487.13±3301.32 CFU/m³ and 2398.79±918.34 CFU/m³ during 2007, 2008 and 2009, respectively. A significantly higher count of fungi was recorded during 2008 ($P=0.0019$) and 2009 ($P=0.0007$) in comparison to 2007. Statistically non-significant difference in fungal count was observed between 2008 and 2009 ($P=0.06995$). The monthly distribution pattern of fungi at different locations is as given in Fig. 1. The total fungal bioaerosols concentration during February and March was higher than the other months. The lowest fungal concentration was observed during May and June.

Among the different sampling sites, the mean fungal content was 2687.86±1362.5 CFU/m³ (750-7363 CFU/m³) at

GAR site, 2306.14±843.88 CFU/m³ (725-4125 CFU/m³) at MAR site, 2211.39± 910.84 CFU/m³ (550-4225 CFU/m³) at RLY site and 1722.72±718.23 CFU/m³ (638-3788 CFU/m³) at JAH site. The fungal count at JAH was significantly lower from GAR, RLY and MAR sites ($F=5.803$, $df=35$, $P<0.001$). The highest aerofungal count was observed during winter (2690±523.75 CFU/m³) followed by monsoon (2076.67±625.26 CFU/m³) and lowest during summer (1929.42±575.63 CFU/m³). The fungal count during winter was significantly higher in comparison to summer ($P=0.0002$) and monsoon ($P=0.0016$). While no significant difference was observed between monsoon to summer season ($P=0.5131$).

3.2 Aerofungal Diversity

In the present study, a total of 41 fungal species belonging to 21 genera were identified from all the four sampling sites. Maximum numbers of fungal species were isolated from MAR site (30 species, 16 genera) followed by GAR site (24 species, 13 genera), JAH site (20 species, 12 genera) and RLY site (19 species, 13 genera) sites. *Alternaria*, *Aspergillus*, *Cladosporium* and *Curvularia* were dominant genera recovered from all the locations. Four genera including *Fusarium*, *Penicillium*, *Phoma* and *Trichothecium* were detected from at least three sites as shown in Table 3. Several allergy causing or toxin producing fungal species including *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus niger*, *Penicillium citrinum*, *Penicillium chrysogenum*, *Fusarium moniliforme*, *Trichoderma harzianum* and *Rhizopus stolonifer* were identified from the air samples as shown in Table 3.

3.3 Effect of Meteorological Factors

A significant negative correlation of airborne fungi was found with temperature ($r = -0.434$ to -0.552) during the study as shown in Table 2. The wind speed also showed significant negative correlation ($r = -0.372$ to -0.379) with fungi concentration. Statistically non-significant correlation was observed with relative humidity (Table 2).

Table 2. Correlation matrix (Spearman's correlation) between meteorological factors and fungal concentrations during 2007 to 2009

	2007	2008	2009
Temperature	-0.552***	-0.456**	-0.434**
Wind Speed	-0.379**	-0.499***	-0.372**
Relative Humidity	-0.023	-0.253	-0.055

** $p<0.01$; *** $p<0.001$

4. DISCUSSIONS

Fungi are omnipresent in the air and are among the most common organisms correlated with air pollution that have adverse effects on human health causing several allergenic diseases. Hence, distribution of fungi in a given environment can be especially important in the diagnosis and treatment of

Table 3. Percent contribution of fungal species at four different locations during different seasons

Species	Summer				Winter				Monsoon			
	JAH	RLY	GAR	MAR	JAH	RLY	GAR	MAR	JAH	RLY	GAR	MAR
<i>Acremonium murorum</i>	-	-	-	1.75	-	-	2.2	-	-	-	-	3
<i>Acremonium roseum</i>	-	-	-	0.88	-	-	1	-	-	-	-	2
<i>Alternaria alternata</i>	21.9	11.34	30.12	21.05	16.5	8.5	20	20.5	11	30	15	20
<i>Alternaria tenuissima</i>	-	-	-	2.63	-	-	2.75					1.5
<i>Aspergillus flavus</i>	25.71	6.19	1.2	-	2.5	8	1	-	24	10	5.5	-
<i>Aspergillus niger</i>	1.9	4.12	-	5.26	0.5	6.12	-	8.26	0.75	3	1.2	4.5
<i>Chaetomium indicum</i>	-	3.09	-	-	-	4	-	-	-	8	-	6
<i>Choenophora cucurbitarum</i>	-	1.03	-	-	-	2	-	-	3	4.5	2	6
<i>Cladosporium cladosporioides</i>	5.71	13.4	14.46	10.53	3	8.5	10.2	6	4	4.5	3.5	2
<i>Cladosporium oxysporum</i>	2.86	7.22	1.84	7.89	2	8.6	12	6.5	3	2.5	2.5	1.75
<i>Cladosporium sphaerospermum</i>	-	5.15	3.61	1.75	0.75	4.15	2.8	1	0.85	4.15	3.25	1.75
<i>Curvularia brachyspora</i>	0.95	-	-	-	1.25	-	0.5	0.5	1.2	1.5	0.75	1.2
<i>Curvularia clavata</i>	-	-	-	1.75	-	-	-	2	-	-	-	-
<i>Curvularia fallax</i>	-	2.06	-	-	-	-	3	-	-	-	-	-
<i>Curvularia lunata</i>	12.38	6.19	6.02	1.75	4	5.6	3.5	3	2.75	2	1.85	3
<i>Curvularia pallescens</i>	4.76	-	-	0.88	3	-	-	1	-	-	-	1.75
<i>Drechslera australiensis</i>	-	-	-	3.51	-	-	-	5.5	-	-	-	4
<i>Drechslera rostrata</i>	-	-	-	3.51	-	-	-	3.75	-	-	-	2.5
<i>Drechslera tetramera</i>	-	2.06	-	-	-	4	-	-	-	0.75	-	-
<i>Epicoccum purpurascens</i>	-	-	-	0.88	-	-	-	3	-	-	-	2.5
<i>Fusarium equiseti</i>	-	-	-	1.75	-	-	-	2.75	-	-	-	2
<i>Fusarium moniliforme</i>	-	-	4.82	2.63	-	-	5	4	-	-	2	1
<i>Fusarium pallidoroseum</i>	3.8	-	2.4	5.26	4	-	3.5	6	-	-	-	2.5
<i>Fusarium solani</i>	-	-	7.23	8.77	-	-	8.5	9.75	-	-	7	6.5
<i>Macrophomina phaseolina</i>	-	-	-	0.87	-	-	-	2.5	-	-	-	2
<i>Neosartoria fischeri</i>	-	2.06	-	-	-	3.25	-	-	-	2.5	-	-
<i>Penicillium citrinum</i>	0.95	3.09	-	-	1.25	4.75	-	-	0.75	1.25	8.4	-
<i>Penicillium chrysogenum</i>	-	-	3.61	3.51	-	-	-	4.5	-	-	-	2.5
<i>Periconia saraswatipurensis</i>	0.95	-	-	-	1.25	-	-	-	2.25	-	-	-
<i>Pestalotiopsis versicolor</i>	-	1.03		0.88								
<i>Phoma eupyrrina</i>	-	-	-	1.75	-	-	-	2.25	-	-	-	2.5
<i>Phoma glomerata</i>	0.95	-	-	0.88								
<i>Phoma jolyana</i>				0.88								
<i>Phoma sorghina</i>	-	-	1.03	-	-	-	2.75	-	-	-	-	0.75
<i>Pleospora multirostrata</i>	3.8	-	-	-	-	-	5	-	-	-	4.2	-
<i>Rhizopus stolonifer</i>	-	-	2.4	-	1.25	2	3.5	-	-	1.5	-	-
<i>Rhizopus oryzae</i>	-	8.25	-	-	4.5	-	2.2	-	2	-	-	-
<i>Stachybotrya atra var. microspora</i>	-	1.03	-	-	-	2	-	-	-	-	-	-
<i>Trichoderma harzianum</i>	0.95	-	-	-	2	-	-	-	-	-	-	-
<i>Trichoderma viridae</i>	1.9	-	-	0.88	2.5	-	-	4	-	-	2	1.75
<i>Trichothecium roseum</i>	6.67	17.53	-	3.51	8	20.75	-	2.25	-	-	2.75	1

various allergic diseases. In this study, total fungal concentration was higher during 2008-2009 in comparison to 2007 as shown in Fig. 1. Total fungal count was also higher from previous studies performed in the urban environment of Delhi, India¹³, and in the suburban and rural environments of West Bengal, India^{14,15}. The mean yearly fungal concentration in this study was higher than the fungal counts in other cities of the world^{8,16-18}. There may be considerable variations between microbial load recorded from different sites within the cities, between cities, rural and urban environment, hour to hour at same location and indoor to outdoor^{8-9,16-20}. These variations in viable count may be due to the selection of site (geography, climatic condition, availability of source), sampling time (diurnal variation), sampling duration (affect fungal viability), meteorological factors (affect release, dispersion, survival and downward settling), type of air sampler and culture media used in the study^{7,17,21}. The maximal load of fungal bioaerosols was found at GAR, the site associated with vegetation. Previous studies also indicated that vegetation contributes towards most of the mycoflora load in surrounding atmosphere as several saprophytic and parasitic fungi can grow easily on plant surface^{1,8}.

The airborne fungi showed a seasonal distribution pattern according to environmental conditions at a given locality^{19,22}. In tropical and subtropical regions, higher fungal load was observed in winters and lower in summers²³. In this study also, fungal count was significantly higher during winter than count in summer and monsoon. During seasonal reversal of wind system (monsoon) the load of fungal bioaerosols was higher from the load of summer but the difference was statistically non-significant (Fig. 1). Significantly higher load during winters may be due to effect of climatic conditions and meteorological factors. The winter meteorological factors, a temperature of 20° C to 27° C and humidity support the growth, dispersal and persistence of the mycoflora in atmosphere (Table 1). The lowest fungi count was recorded during extreme summer. This could be due to high temperature in this area which is unfavorable for the growth of plants as well as fungus²⁴. The fungal bioaerosols during monsoon (a rainy season), was observed lower than winter which was quite surprising. This would be associated with rainfall as effect of rainfall is interesting, rain stimulates fungal growth in one side, while on the other removes air borne fungi by rainout capture (involving fungi as condensation nuclei, which fall with the resultant droplets) and washout effects²⁵. High relative humidity during rainy season also makes spores to absorb water, making them heavier and less transportable by air²⁶. A negative correlation was observed between wind speed and fungal count (Table 2). This could be due to the dilution effect in fungi because of dispersal of fungal spores due to wind speed⁶. The non-significant effect of humidity can be explained as moisture in atmosphere stimulates fungal growth but their dispersal in air is retarded at higher humidity.

Data calculated on percentage of different fungal species of Gwalior revealed that the species of *Alternaria*, *Aspergillus*, *Curvularia* and *Cladosporium* were dominated throughout the year as shown in Table 3. *Penicillium* was also frequently isolated but its percent contribution was lower in total concentration. Many of the species identified in the atmosphere

like *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus niger*, *Cladosporium cladosporioides*, *Drechslera*, *Epicoccum*, *Fusarium*, *Penicillium citrinum*, *Penicillium chrysogenum*, *Rhizopus*, *Stachybotrya* and *Trichothecium* sps are known to cause variety of adverse health effect via pathogenesis, allergies and production of mycotoxins²⁷. In Indian subcontinent, more than half of viable airborne fungi were found allergenic in skin prick test^{14,15}. The daily fungal spore concentration is correlated with increase in number of emergency visit and hospital admission due to exacerbation in asthma attacks²⁸.

In conclusion, during this study, fungal concentrations showed variations according to the site, month, season and year of sampling. The maximum count was observed at garden (GAR) and lowest at the hospital (JAH). Season wise, lowest concentration was found in summer, higher in monsoon and maximum in winter. Among the meteorological factors studied, temperature and wind speed showed significantly negative correlation with the fungi concentration. The major dominating genera present in the air were *Alternaria*, *Aspergillus*, *Curvularia* and *Cladosporium*. Some genera like *Cladosporium* and *Alternata* are highly allergenic and may cause a variety of health problems. Hence, it is very important to monitor the concentration and type of airborne fungi in the environment for evaluation of human health risk.

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CONTRIBUTORS

Dr Pramod Kumar received his PhD (Biotechnology) from Jiwaji University, Gwalior, in 2010. He worked as CSIR-Senior Research Fellow at DRDE, Gwalior from 2007-2010. Presently, he is working as Scientist-III at All India Institute of Medical Sciences, New Delhi. His research is focused on mechanism of antimicrobial resistance and virulence in microbes.

Dr Ajay Kumar Goel received his PhD (Microbiology) from CCS Haryana Agricultural University, Hisar, in 1999. Currently working as a Scientist 'F' and Head, Bioprocess Technology Division, Defence Research and Development Establishment, Gwalior. He has more than 90 research paper, 6 patent, radio talks, and several overseas presentations to his credit. His current interests include : Development of detection and protection systems for potential biothreat agents.