Asian Pacific Journal of Allergy and Immunology (1993) 11 : 19-28

Survey of Airborne Culturable and Non-Culturable Fungi at Different Sites in Delhi Metropolis

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SUMMARY **A two year aerobiological survey for culturable and non-culturable fungi was conducted at human height at five different sites in Delhi metropolis. Burkard Personal Volumetric Sampler for petriplates and slide exposures were used for sampling the air. With simultaneous petriplate and slide exposure a total of 98 fungal forms were recorded.** *Cladosporium* **contributed for 25-40% of total airborne fungi followed by** *Ustilago* **(smuts) (24%)** *Aspergillus flavus* **(10-13%),** *Alternaria* **(11%) and** *A. niger* **(8%). Basidiomycetes contributed 7-13% at different sites. The frequency of occurrence of these types varied from 50-98%. In general fungal concentration was high from July to April with low counts in winter (January) and dry and hot summer (May-June). Quantitative variations in the spore counts were found to be statistically significant within the same urban locality.**

Fungi play an important role in nasobronchial allergy as is evident by from the literature in the field during the last two decades.[1-4] For effective diagnosis and therapy of mold allergy, information on their qualitative and quantitative prevalence and variations in both outdoor and indoor environments is of immense value.[5] Aerobiological survey for fungal spores was carried out in Delhi in 1969 by Agarwal and Shivpuri in which dominant fungal types were recorded based on gravity settling method conducted at one site alone.[6] Later Singh and Babu carried out a volumetric survey for a brief period of six months to find out the circadian periodicity of certain fungi.[7] However, with rapid changes due to urbanization and industrialization affecting the vegetational cover, the composition of airborne fungi would have changed considerably during the past two decades.

As the previous survey was of qualitative nature and carried out two decades ago the quantitative information on airborne fungi in Delhi area has long been overdue. The present volumetric survey was therefore, undertaken with the objective to find out the qualitative and quantitative variations of the culturable and non-culturable airborne fungi in different ecozones of Delhi metropolis.

MATERIALS AND METHODS

Delhi, with an area of 1,483 km^2, is situated between 28° 12′-28° 53′ N and 76° 50′-77° 23′ E and is the seat of Government of India. The city is inhabited on both sides of the river Yamuna, flowing from North to South. Aravalli hillocks (Delhi ridge), which support natural scrub forest, also run from north to south. Besides, the city has exotic plantation in gardens, along the road sides and is surrounded with vast agricultural tracks.

The aerobiological survey for airborne fungi was carried out for two consecutive years from October, 1989 to September, 1991. For the purpose of sampling, the city was arbitrarily divided into five zones, representing North, South, East, West and Central zones (Fig. 1). Sampling was carried out using Bur-

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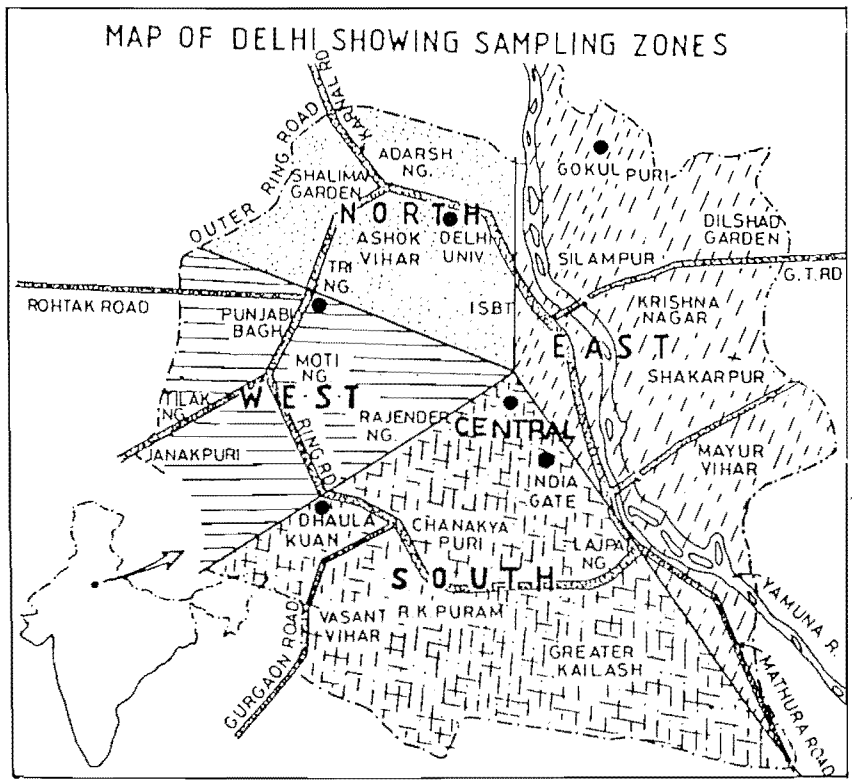


Fig. 1 Different sampling sites selected for fungal survey during 1989-90 and 1990-91 in Delhi metropolis.
North : North campus Delhi university
South : South campus Delhi university
East : Trans Yamuna residential locality
West : Lawrence road industrial area
Central : Cannuaght place official/shopping centre

kard personal petriplate sampler (Fig. 2a) for culturable fungi, and Burkard slide sampler for non-culturable fungi (Fig. 2b). The samplers were procured from M/s Burkard Manufacturing Co, UK. These samplers are designed for isokinetic sampling and are independent of wind speed and direction. Samples were collected at 5'-6' height from the ground level at a 10 day regular intervals. The sampler was operated each time for 3 minutes for petriplate and 10 minutes for slide, between 10-12 noon. Rose Bengal Sabouraud's agar medium was used for petriplates and Glycerine jelly for coating microslides. Exposed petriplates were incubated at 27 + 2°C for 72 hours and the developed colonies were isolated and identified based on colony characteristics and literature.[8,9] The exposed slides were mounted and scanned microscopically for various spore types.

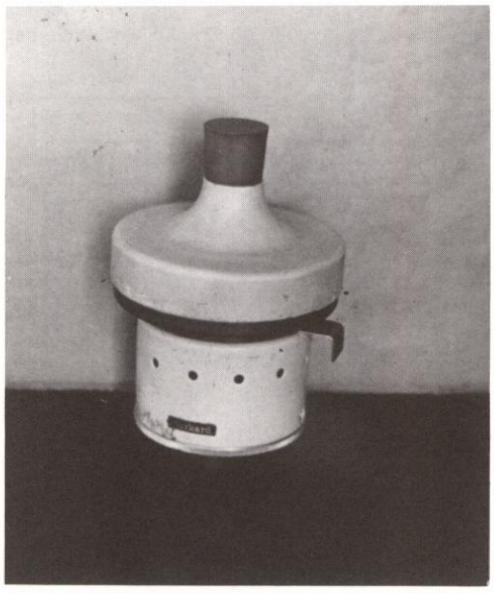
All the colony counts obtained were expressed as colony forming units per cubic meter (CFU/m^3) and spore counts as spores per cubic meter (spores/m^3) of the air sampled. The extent of variation in the concentration of total fungi from different experimental sites was statistically analysed using analysis of variance.

RESULTS

Qualitative prevalence

During the present investigation a total of 98 fungal types were isolated and identified from the air of Delhi. Of these 69 belonged to Deuteromycetes (Fungi imperfectii), 7 each to Ascomycetes and Zygomycetes and 14 to Basidiomycetes. Spores of *Ustilago* spp. were counted separately as smuts.

The percent contribution of 10 dominant fungal forms along with their percentages of occurrence on petriplates and slides can be seen



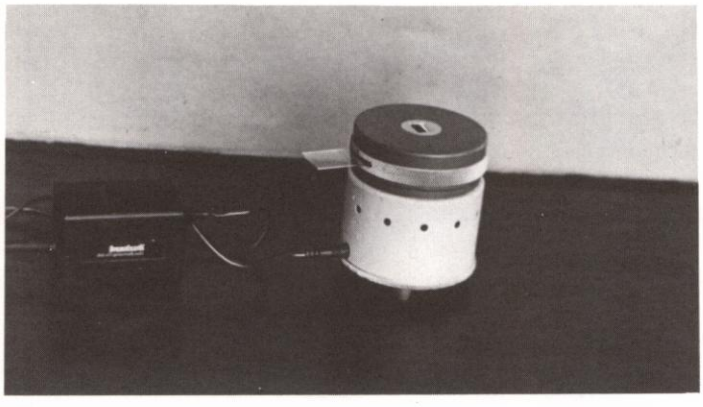


Fig. 2a. Burkard Personal Volumetric Sampler (Petriplates)
2b. Burkard Personal Volumetric Sampler (Slides)

from Table 1. *Cladosporium, Aspergillus flavus, Alternaria, Aspergillus niger* and Basidiomycetes contributed significantly, varying from 8% to 40% to the total aeromycoflora. These were observed on more than 90% of the days sampled, except for *Ustilago*(smuts) which had about 80% occurrence. Fungal types which were frequently recorded with less than 1% contribution were *Cunninghamella, Absidia, Paecilomyces, Fusarium, Pithomyces Aspergillus glaucus, A. janus, A. ustus* and others.

Monthly concentration

The monthly concentration of the fungi together (total) and of the dominant types, recorded at different sites during 1989-90 and 1990-91, are graphically presented in Figs. 3-10.

The concentration of total fungal colonies (CFU/m^3) and visual spore counts (spores/m^3) were variable in each month, but the period from October to January was characterised by high fungal concentration with peaks in December and January (Figs. 3,4). *Cladosporium* although present in each month was predominantly high from October to February in both years from each site (Fig. 5). The *Ustilago* (smuts) showed distinct pattern in both the years with high concentration from January to May (Fig. 6). Colony concentration of *Alternaria, Aspergillus flavus* and *Aspergillus niger* varied from month to month but showed seasonal excerbations. *Alternaria* was as high as 978 CFU/m^3 in April (Fig. 8) whereas the peaks from *Aspergillus flavus* and *Aspergillus niger* were in October and September, respectively (Figs. 7, 9). The spores of basidiomycetes, other than *Ustilago* (smuts) were recorded in low concentration from October to June at all sites. (Fig. 10).

Site to site and yearly variations

Annual concentration of predominant culturable and non-culturable fungi recorded during the two year survey, from different sites of Delhi are presented in Table 2. Year to year and site to site variations in their concentration were observed. For example, the average annual colony concentration of *Epicoccum* were only 644 CFU/m^3 in 1989-1990 but the concentration increased to 1,273 CFU/m^3 in 1990-91. Similarly, *Cladosporium* was as high as 9,196 CFU/m^3 from south zone, whereas only 5,257 CFU/m^3 were recorded from the west zone in the same year. The other fungi also exhibited variations in their concentration from one site to other and also from year to year.

Total spore concentration, recorded by slide counts from different sites when statistically analysed for site to site variation was found to be significant at five percent level ($p < 0.05$).

DISCUSSION

Continuous monitoring of aeroallergens is desirable due to changing vegetational and crop pattern. Not

Table 1 Percent contribution of dominant culturable and non-culturable fungi and their percent occurrence during 1989 - 1991.

		Contribution %		Occurrence %	
Fungal Types	Year	CFU/m^3	Spore/m^3	CFU/m^3	Spores/m^3
Cladosporium	89-90	39.04	27.46	67.57	95.23
	90-91	42.62	25.04	66.11	91.77
Ustilago	89-90	*	24.03	*	78.64
(smuts)	90-91	*	24.12	*	79.34
Aspergillus flavus	89-90	13.17	**	76.97	**
	90-91	10.37	**	84.11	**
Alternaria	89-90	11.22	11.67	59.05	94.61
	90-91	9.56	10.51	50.58	97.64
Aspergillus niger	89-90	8.79	**	75.61	**
	90-91	8.84	**	86.19	**
Basidiospores	89-90	*	7.80	*	91.13
	90-91	*	13.19	*	97.03
Aspergillus japonicus	89-90	3.24	**	46.45	***
	90-91	3.21	**	35.63	**
Drechslera	89-90	2.11	1.96	23.94	75.98
	90-91	1.06	1.16	18.58	62.31
Ascospores	89-90	*	1.60	*	65.87
	90-91	*	1.49	*	64.17
Epicoccum	89-90	3.31	1.17	23.05	36.30
	90-91	4.96	1.05	20.09	31.06

* = Non-culturable type recorded only from slides.
** = Culturable type recorded from petriplates.

Table 2 Average annual concentration (No/m^3) of dominant fungi during 1989-90 and 1990-91.

		Different ecozones					
Types of fungi	Year	Nonth	South	East	West	Central	Average annual concentration
Cladosporium	89-90	9275	9196	8716	5257	5503	7589.4
	90-91	7467	9530	7521	6334	6893	7549.0
Aspergillus flavus	89-90	2221	2320	1762	4420	2084	2561.4
	90-91	1941	1337	1494	3098	1318	1837.6
Aspergillus niger	89-90	1318	1115	2029	1285	2797	1708.8
	90-91	1345	1181	1813	1497	1682	1503.6
Epicoccum	89-90	1006	715	1049	156	294	644.0
	90-91	723	1435	621	465	843	1273.0
Penicillium oxalicum	89-90	1398	892	0000	452	1216	791.6
	90-91	400	912	410	597	953	654.4
Ustilagu	89-90	2946	13348	2404	3757	6099	5711.2
	90-91	4759	6288	4594	3448	3341	4486.0
Alternaria	89-90	2149	2619	2648	2656	1979	2410.2
	90-91	2281	2148	2393	1114	1831	1053.4
Aspergilli penicilli	89-90	2176	1949	1754	5534	2443	2771.2
	90-91	3714	2893	1581	4874	1759	2964.2
Ascospores	89-90	178	318	304	504	349	330.6
	90-91	183	399	176	329	299	277.6
Basidiospores	89-90	1781	2501	1553	1003	1216	1610.8
	90-91	1456	7539	1556	844	859	2450.8

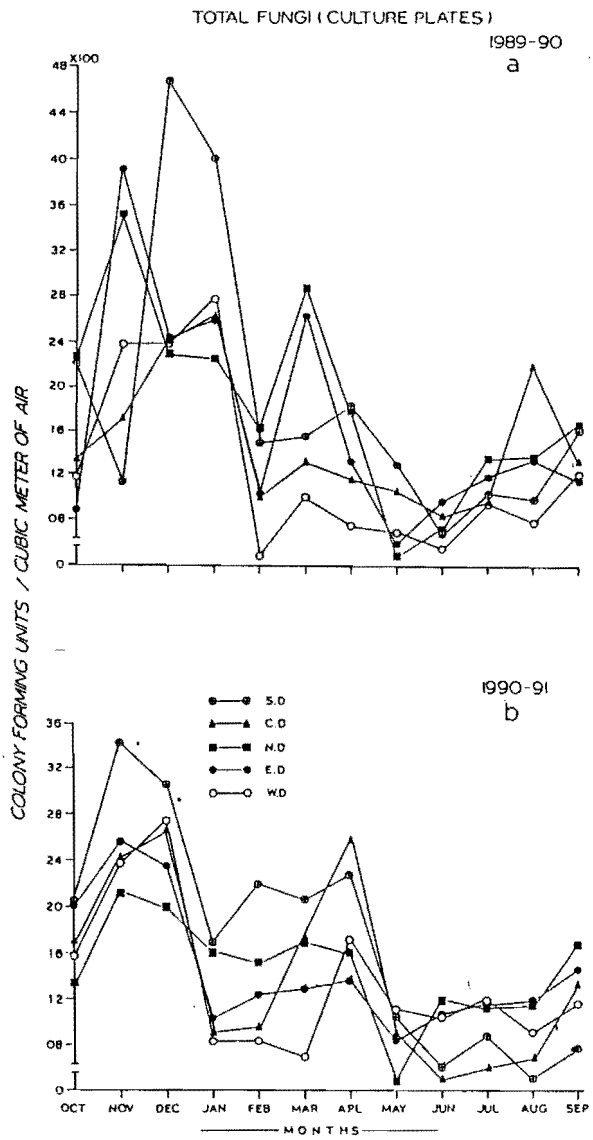


Fig. 3 Monthly concentration of total fungi (CFU/m^3) as recorded from petriplate exposure from five different sites. a : 1989-90 b : 1990-91

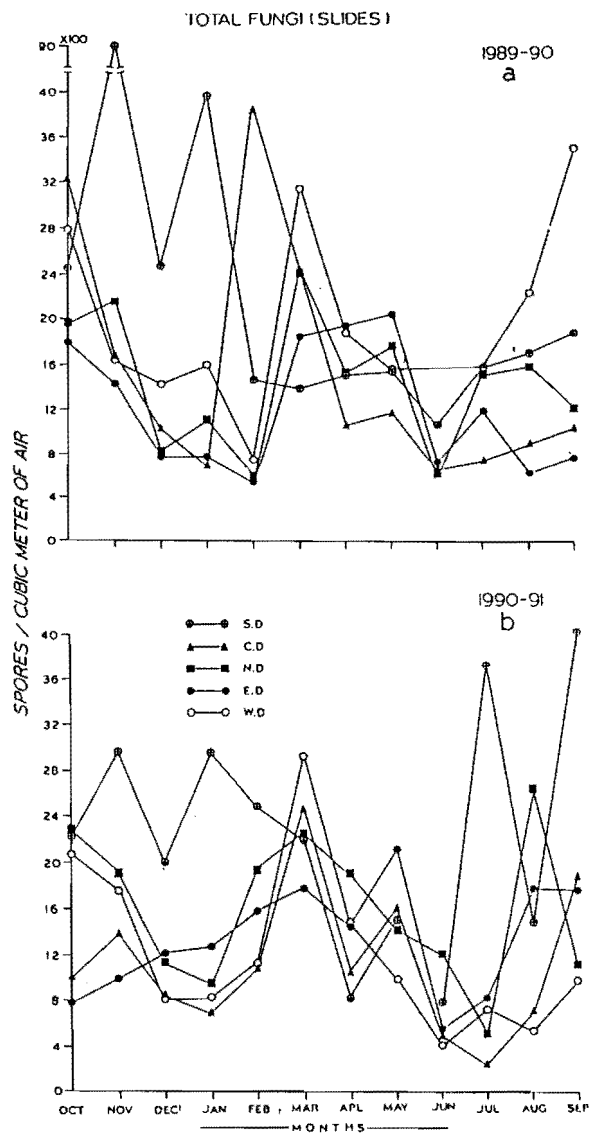


Fig. 4 Monthly concentration of total fungi (spores/m^3) as recorded from slide exposure from five sites. a : 1989-90 b : 1990-91

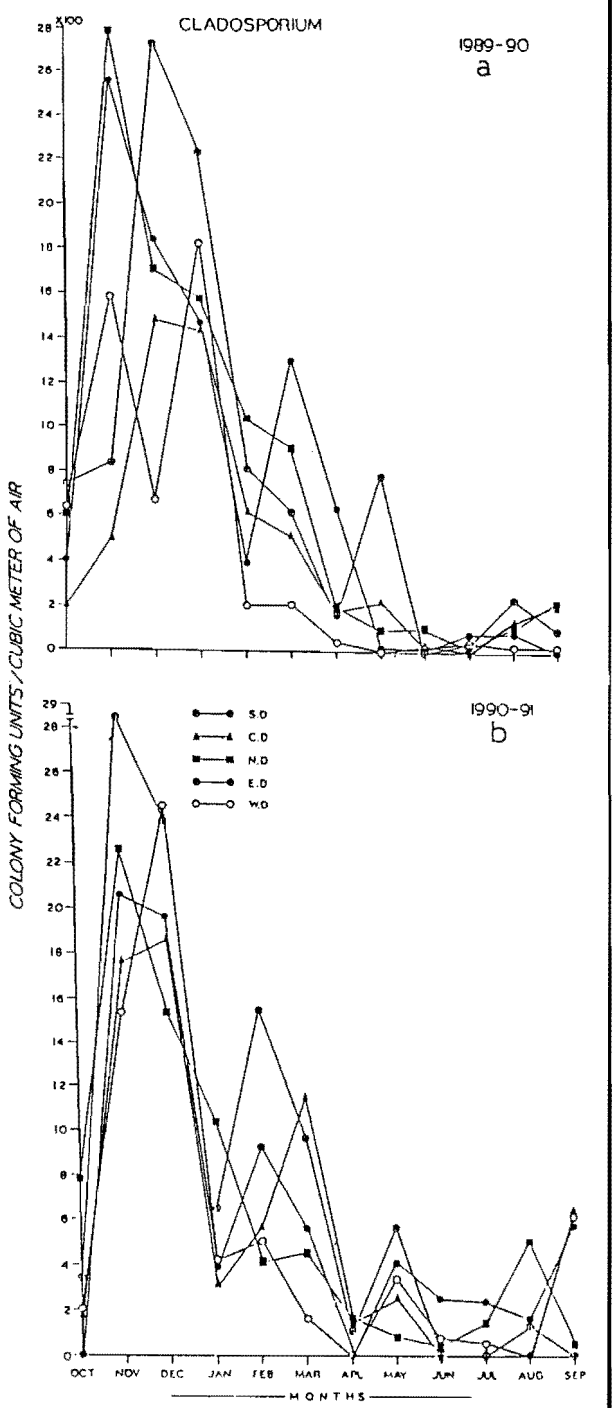


Fig. 5 Colony concentration of *Cladosporium* (CFU/m^3) as obtained in different months during investigation. a : 1989-90 b : 1990-91

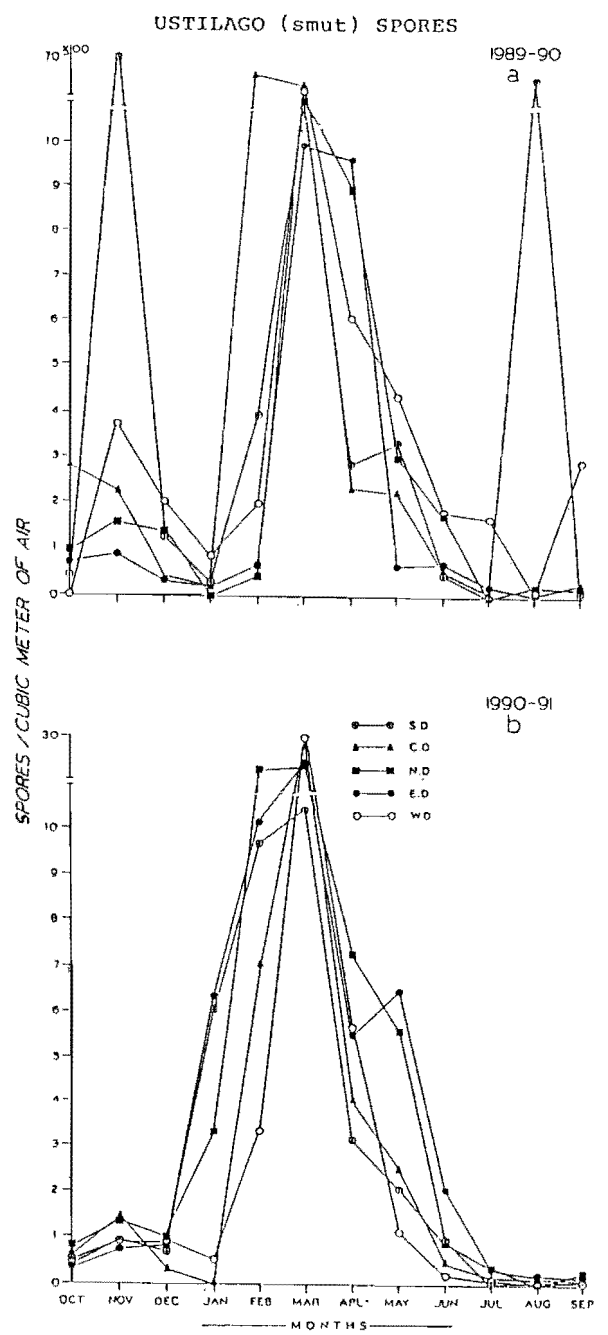


Fig. 6 Spore concentration of *Ustilago* (smuts) (spores/m^3) in different months as recorded from various sites of exposure. a : 1989-90 b : 1990-91

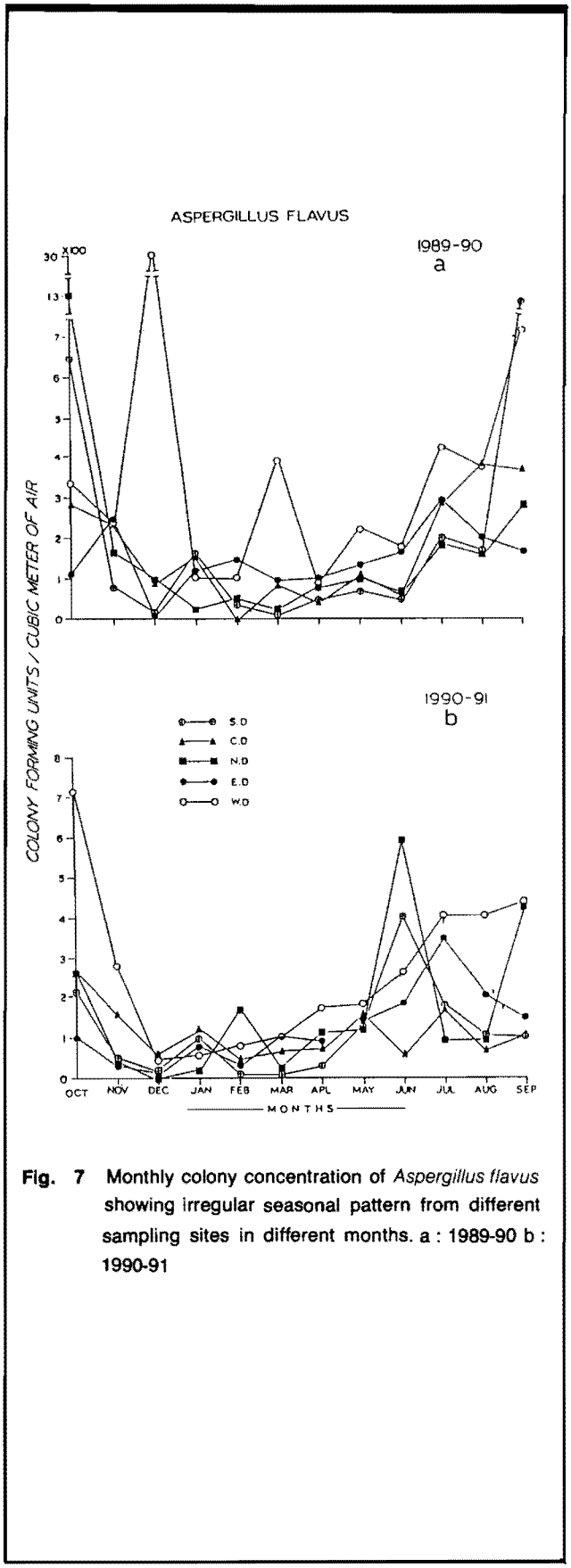


Fig. 7 Monthly colony concentration of *Aspergillus flavus* showing irregular seasonal pattern from different sampling sites in different months. a : 1989-90 b : 1990-91

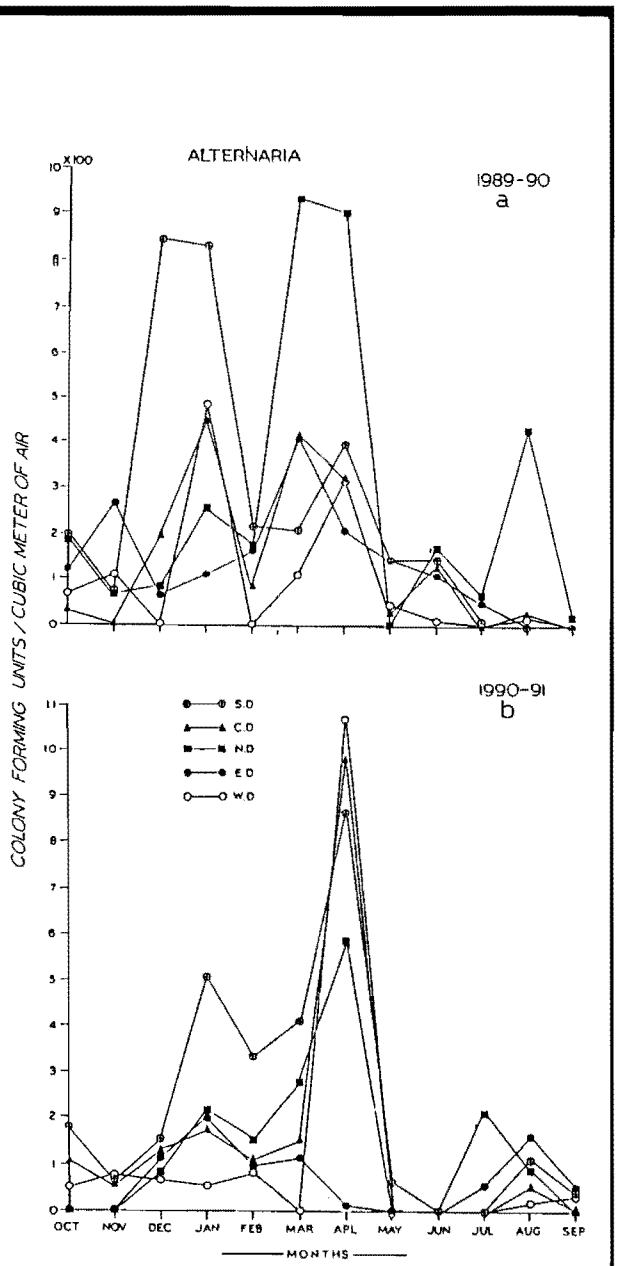


Fig. 8 Colony concentration of *Alternaria* in different months showing high recovery during March-April. a : 1989-90 b : 1990-91

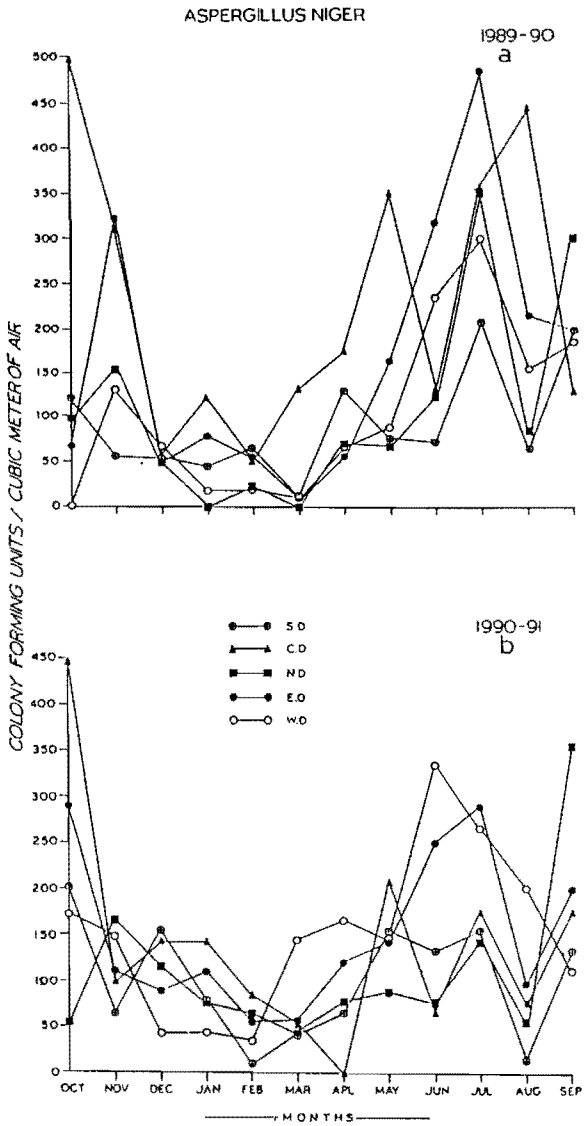


Fig. 9 Monthly colony concentration (CFU/m^3) of *Aspergillus niger* showing indistinguished seasonal occurrence. a : 1989-90 b : 1990-91

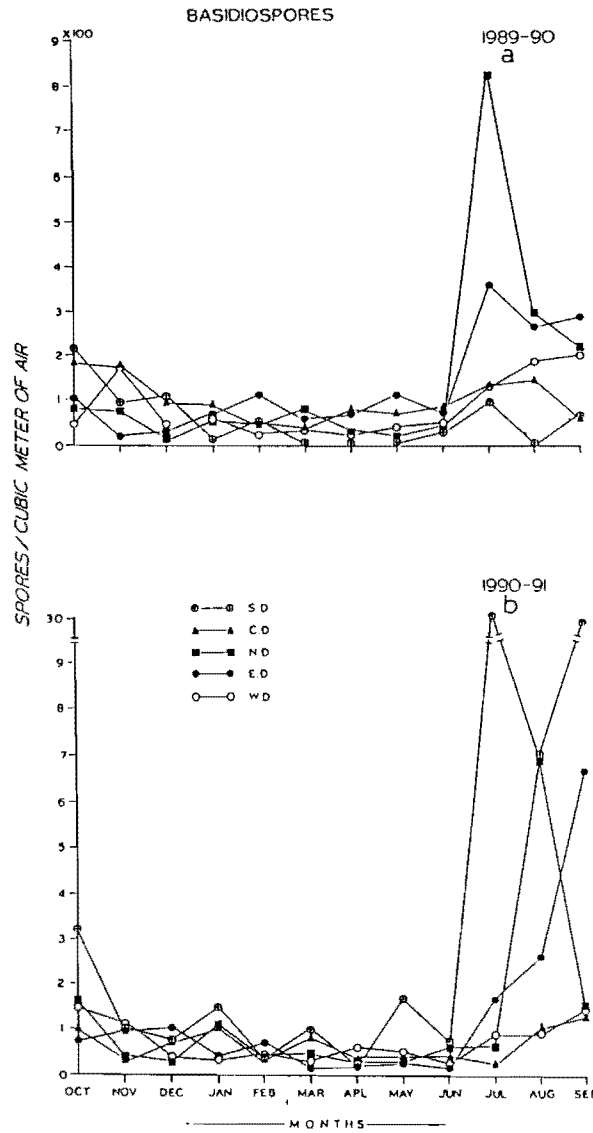


Fig. 10 Spore concentration of Basidiomycetes (spores/m^3) recorded from different experimental sites in Delhi. a : 1989-90 b : 1990-91

only the city surroundings but also the different local environment inside a city affect the fungal flora. The application of culture plate and slide sampler together in different localities therefore gave a better qualitative and quantitative spectrum of fungi in the atmosphere of Delhi. Sampling was carried out at human height in order to reduce the background counts at higher levels and to assess the effect of the sources which are nearer to the ground. The sampling around the midday was selected considering that at that time maximum population is exposed to outdoor environment.

Of 98 fungal forms recorded, the dominant fungi isolated were *Cladosporium, Ustilago* (smuts), *Aspergillus flavus, Aspergillus niger,* Basidiomycetes, *Aspergillus japonicus, Drechslera* with ascospores and *Epicoccum*. Agarwal *et al*[6] in their single point survey reported *Alternaria* as the predominant fungal form in Delhi whereas in the present survey *Cladosporium* were found to be the most dominant fungi invariably in all the sampling zones irrespective of the sampler used. *Ustilago* contributed 20-24% to the total airborne fungi whereas earlier reports from Delhi and other parts of India revealed only 5-15%. Higher atmospheric concentration of *Ustilago* have also been reported by Hasnain and Collins-Williams from New Zealand and Canada respectively.[10,11] Some of the fungal forms such as *Dictyostelium, Polysphodylium, Piptocephalis, Aspergillus carbonarius, Chrysosporium* and *Penicillium wortmanni* reported earlier from Delhi[6] could not be isolated during our survey but in addition spores of Basidiomycetes and some of the Ascomycetes are observed for the first time in the air of Delhi. In view of the allergenic significance of Basidiomycetes[12] the current survey suggest that these could be potential aeroallergens in India. Other dominant fungi observed in our survey have also been recorded in other parts of India, Asia, Europe and USA.[10,13-20]

Other commonly encountered types were *Penicillium* spp., *Fusarium, Rhizopus, Aspergillus* spp., *Nigrospora, Trichoderma, Paecilomyces, Candida, Humicola, Cercospora, Sporormia, Tetraploa,* and *Torula*. These qualitative differences were possibly due to wide spread volumetric sampling conducted for culturable and non-culturable fungi. The combination of both the techniques and other parameters is also emphasised by Solomon[21] and Burge.[22]

Delhi, in general, is climatologically characterised by three major seasons, spring (mid February - mid April), summer (mid April - end of June) and winter (mid October-mid February). The period from July to mid October is of precipitation. We found that the fungal concentration was high from July to April which could be due to moderate to high humidity, optimum environmental temperature and increased wind currents which help the spores to become airborne. The spore load was found low in winter (January) and extremely low in dry and hot summer months (May and June). Similar observations were also made by Aldoory.[2] *Cladosporium,* the most predominant form, showed high concentration in November and December when the climate is dry with a low mean temperature of 15°C-25°C. The spore counts were also high during March when temperature rises to optimum and high velocity wind currents agitate the withering plant materials and dead fallen leaves which act as a reservoir to this fungi. The low counts on petriplates in dry summer suggest the probable loss of spore viability due to high atmospheric temperature. Similar observations have also been made by Burge.[22]

Ustilago spores appear in November when temperature and wind velocity are moderate to high. In spring (March-April) *Ustilago* are airborne due to repeated lawn mowing and disturbance of infected wheat crop in the fields in and around the city.

Although the coloured and hyaline basidiospores were recorded throughout the year on 90-95% of the days sampled, high concentration was observed from July to October. It could be due to high atmospheric salt and water content with optimum temperature which promotes and supports the growth of various types of mushrooms and puffballs in lawns, garden trees and in forests. Of the 14 different types identified, *Ganoderma* and *Cantharellus* had distinct seasonal pattern predominantly during August and September. The *Coprinus, Russula, Conocybe, Agrocybe, Psathyrella, Fomes* were observed sporadically in the air. The high prevalence of different Basidiomycetes in the air has been reported from other parts of the world.[23-25]

The sampling at 5 different sites within the metropolis revealed significant quantitative variations. *Alternaria, Cladosporium, Drechslera* and basidiospores were observed in higher concentrations in south and north zones as compared to other zones. This could be due to these zones being characterised by more parks, lawns and scrub forest on Delhi and New Delhi ridge. Vegetational cover in these localities act as a reservoir for the above types which thrive on dead and decaying plant parts and trees. Smuts and *Aspergillus niger* were high from east zone which is surrounded by agricultural fields, farms and has extensive slums of Delhi. The latter is a characteristic fungi of such environment and have been recorded from this zone. The high concentration of *Aspergilli* from west zone could be due to industrial surroundings such as bakery, cold storage and vegetable and fruit processing units.

ACKNOWLEDGEMENTS

Authors wish to acknowledge

the technical help made by Mr Sunil Dutt Attrey and partial financial assistance from Ministry of Environment and Forests, Government of India, New Delhi, India.

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