

Airborne fungal spores in an industrial area: seasonal and diurnal periodicity

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Abstract

Qualitative and quantitative studies of atmospheric fungal spores at a chloralkali factory, Jayashree Chemicals, were made during 1993 employing culture plate and rotorod methods. A total of 57 sporulating fungal types, including three sterile mycelial forms, were recorded by the culture plate method and 51 spore types, including the hyphal fragments and unidentified spores, were recorded by the rotorod method. As to the seasonal variation, winter was found to be the greatest contributor of fungal spores as compared to the summer and rainy season. Instead, when considering the hour of the day, the peak number of fungal propagules was recorded at noon (12.00 h) followed by evening and morning values, an exception being recorded in winter months, when maximum CFUs of *Cladosporium* were monitored in the morning. The seasonal variation in fungal concentration and composition was found to be influenced by temperature, rainfall and relative humidity, whereas diurnal incidence was the effect of varying temperature and relative humidity during day time only. Moderate temperature and relative humidity favoured the maximum fungal spore load in the atmosphere. *Cladosporium*, *Nigrospora*, *Alternaria*, *Lasiodiplodia*, *Drechslera*, *Pestalotia*, *Curvularia*, *Epicoccum*, *Aspergillus*, *Penicillium* and *Chaetomium* were the commonest fungal spores in the factory area. © 1998 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Chloralkali factory; Fungal propagules; CFUs; Seasonal variation; Diurnal variation

1. Introduction

Airborne fungal spores contribute a major share of bioaerosols and investigations are essential to understand their distribution, ecology and biodeterioration, to forecast plant diseases (Chaubal and Kotamire, 1982) and to detect allergies and skin diseases (Singh and Singh, 1994). The incidences of such diseases are increasing steadily in metropolitan and industrial areas drawing thus considerable attention in recent days (Nayak and Behera, 1996).

The number and type of airborne fungal spores of a locality vary according to the time of the day, weather,

geographical location and presence of the local spore sources. For instance, seasonal crops and vegetation provide compost material favouring the growth and reproduction of large numbers of fungi irrespective of human interference (Hyde, 1972; Kumar, 1984; Fernandez-Gonzalez et al., 1993; Nayak and Behera, 1996). Depending on the concentration and circumstances of exposure, fungal-spores may cause a range of allergic diseases sometimes through infection or more often owing to their immunotoxic properties (Aberg, 1989; Ellis and Gallup, 1989). Therefore, it is necessary to gain knowledge on the ecology of fungal airspora and aeroallergens and on the disease they cause. The present investigation provides data only on seasonal and diurnal incidence of fungal airspora studied at a chloralkali factory.

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2. Materials and methods

The present study was conducted inside the premises of a chloralkali factory, Jayashree Chemicals., located in Ganjam, about 1 km away from Ganjam town and 30 km away from Berhampur city, Orissa, on the coastal belt of the Bay of Bengal (85°3'E longitude, 19°23'N latitude). The factory discharges mercury-based effluents, solid wastes and chlorine gas that are main agents of water, soil and air pollution, respectively. The regular discharge of solid wastes inside the factory premises over the years has almost erased the natural herbaceous and annual vegetation that is sparsely seen around the factory and only patches of *Pandanus fascicularis* and certain tree plants are found inside. The factory area is surrounded by the estuary of the river Rushikulya in the south, the Bay of Bengal in the east and paddy fields in the west and north. The site has a monsoon climate with coastal features, the temperature varies between 15 and 40°C and the annual rainfall never exceeds 1300 mm. Indian meteorologists recognise three seasons, i.e. winter (November–February), summer (March–June) and rainy season (July–October). Rain is mainly due to the south-west monsoon that begins by the middle of June and extends up to the end of September or the 1st week of October with maximum rainfall between 15th July and 15th September.

Air samplings were made during morning, noon and evening hours at fortnightly intervals on the top of a building (10 m above ground level) from January to December 1993 employing the rotorod sampler as well as exposing media plates (Ø 9 cm). Petridishes containing sterile PDA medium with streptopenicillin (50 mg/l) in three replicates were exposed to air for 5 min, the time being standardized to get the requisite number of colony forming units (CFUs) per plate. The exposed Petridishes were incubated in a culture room at $25 \pm 3^\circ\text{C}$ for 7 days with observations performed every day for fast growing colonies. The CFUs in plates were counted and identified up to species level.

Simultaneously, volumetric air sampling was made by operating the rotorod for 1 h. The cellotape stripes were removed carefully, cut to 18 mm length segments and scanned under microscope for ten microscopic fields. Identification of fungal spore types and fungal species was made with the help of standard taxonomic books and available literature (Ellis, 1971; Subramanian, 1971; Barnet and Hunter, 1972; Ainsworth et al., 1973; Gregory, 1973; Gilman, 1975; Von Arx, 1981; Onions et al., 1986; Tilak, 1989). The total number of spores counted were converted to the numbers m^{-3} of air multiplying by '5' (Tilak, 1987) and calculated as follows,

breadth of the trace	2 mm
width of each scan	350 μm or 0.35 mm and 10 scans = 3.5 mm
total area scanned	$3.5 \times 2 \text{ mm} = 0.07 \text{ cm}^2$,
speed of rotation	2000 rpm
duration of sampling	60 min
length of the sweep	$\pi \times \text{diameter} \times \text{rpm} \times \text{time}$ $= 3.14 \times 82 \times 2000 \times 60$ $= 3017, 142.857 \text{ cm}$,
volume of air; scanned	3017, 142.857 $\text{cm} \times 0.07$
within the scanned area	$\text{cm}^2 = 2112200$ $\text{cm}^3 = 0.2112 \text{ m}^3$
conversion factor for	m^{-3} of air = $1/0.2112$
estimating	= 4.73 or 5.

Spores with rounded appearance mostly belonging to some members of aspergilli, penicilli, mucorales and *Gliocladium* could not be identified and were considered as unidentified round spore types. Percentage contribution to the total numbers by an individual spore type or species was determined as the total number of the spores or CFUs of the individual fungus divided by the total number of spores of all the spore types or total number of CFUs of all the fungal species multiplied by 100.

Meteorological parameters like atmospheric temperature and relative humidity recorded at the site were correlated with the fungal spore concentrations taking meteorological observations as independent variables and fungal spores as dependent variables (Pearson's coefficient of correlation). To relate the species composition in diurnal variation, similarity coefficient was calculated using the formula $(2W/a + b) \times 100$ where, W is the number of common fungi, 'a' is the total fungi of one sample and 'b' is the total fungi of the other sample.

3. Results

During the study period, 91 fungal types altogether were recorded by both methods. The total number of CFUs isolated by the culture plate method was 3307, assigned to 57 species. Each fungal colony on agar plate was assumed to be developed from a single viable unit, spore/hyphae. Of the total isolates of the species, members of Deuteromycotina accounted for 98.25% and it was followed by the members of mucorales (1.75%). A total of 7605 fungal spores m^{-3} of air assigned to 51 spore types was recorded by the rotorod. Most of the fungal spore types belonged to Deuteromycotina accounting for 78.43% of the total spores, followed by 19.61% of ascospores and 1.96% of spores of mucorales.

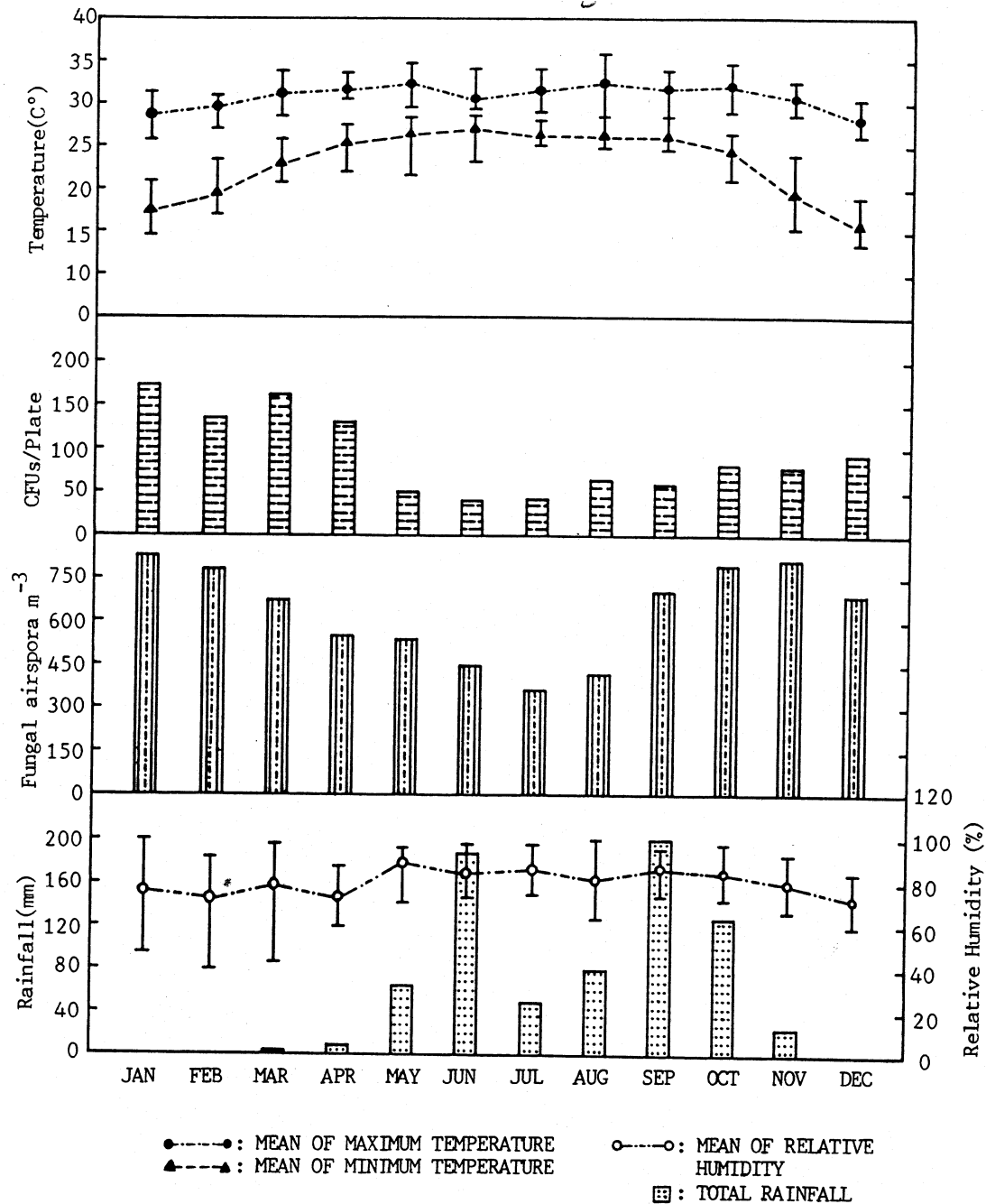


Fig. 1. Monthly variation in fungal spore concentration, total rainfall, mean of relative humidity, mean of maximum and minimum temperatures recorded during 1993.

Monthly incidence of fungal population together with the atmospheric parameters is quite evident from Fig. 1. The maximum number of spores was recorded in January and November and the minimum in June and July. Using the rotorod the highest number of fungal airspora m^{-3} was monitored in winter (November–February) followed by the values of the rainy season (July–October) and summer (March–June). With the culture plate method, the lowest number of fungal spores was isolated during the rainy season (Fig.

2). Diurnal incidence in fungal airspora was studied utilizing both methods. With the culture plate method the highest number of colony forming units was recorded at noon, accounting for 45.8% of the total isolates followed by values referring to morning and evening hours (Fig. 3). Similarly, when studied volumetrically, the highest number of fungal airspora m^{-3} was recorded around noon followed by evening and morning hours contributing 46.4, 29.3 and 24.3% of the daily total, respectively.

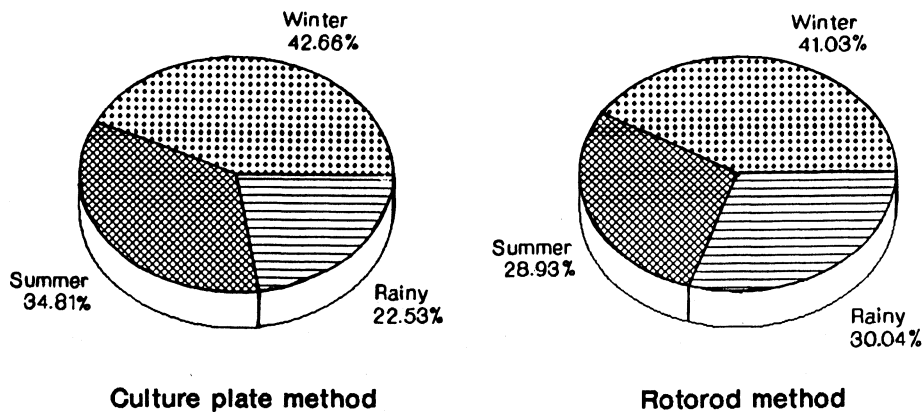


Fig. 2. Seasonal variation in spore concentration.

A relationship in terms of common number of species recorded between the three sampling times is given in Table 1, which clearly revealed that morning time was found to be better related to noon time followed by noon and evening times while, evening and morning times were less related to each other as to their fungal composition.

Airborne fungal spores m^{-3} and meteorological parameters like temperature and relative humidity were statistically correlated, the correlation coefficient values becoming negative (Figs. 4 and 5).

Relative abundance of different fungi recorded by both techniques at morning, noon and evening hours is given in Table 2. Analysis of the culture plate method revealed *Cladosporium cladosporioides* as the dominant fungal spore accounting for 19.28% of the average total, followed by non-sporulated Gray sterile mycelial forms (13.91%) and *C. herbarum* (5.93%). *Cladosporium* mostly occurred in winter months, contributing above 50% to the winter total airspora m^{-3} and it was found to be the fourth dominant one contributing 7.23% to the annual total m^{-3} of air, whereas *Nigrospora sphaerica* was the first dominant one accounting for 15.78% of the average annual total followed by hyphal fragments (9.73%) and unidentified spores (8.22%). On observation of the individual fungus, a few fungal types were recorded throughout the year with peak concentration over different periods, viz. *Alternaria* during February and April, *Drechslera* during April and June, hyphal fragments (August and September), *Nigrospora* (March and September), *Periconia* (May and September) and unidentified spore types (May and August) in other months. However, occurrence of certain fungal types exhibited restriction to seasons being recorded sporadically in other periods of the year; *Cladosporium*, *Fusarium oxysporum*, *Albugo*, *Alternaria padwickii*, *Curvularia*, *Diplodia*, *Sporormia*, *Beltrania*, *Tetraploa* and *Colletotrichum* were predomi-

nant in winter but hardly occurred in late summer and early rainy season whereas, *Pithomyces*, *Chaetomium*, *Memmoniella echinata* and *Paecilomyces* were found mainly during rainy season and *Haplosporella*, *Fusariella*, *Humicola*, *Pestalotia*, *Aureobasidium pullulans* were restricted to summer.

Members of aspergilli, penicilli and mucorales were higher in number during early rainy periods. Besides the dominant ones, contributing substantially to the annual total airspora, a large number of fungal types were recorded incidentally one to three times during the study period, contributing thus negligibly.

Isolates of *Alternaria dianthicola*, *Aspergillus flavus*, *A. fumigatus*, *A. wentii*, *Chalaropels* sp., *Curvularia lunata* var. *aeria*, *C. Oryzee*, *C. pennisetii*, *Drechalera halodes*, *D. hawaiiensis*, *D. phlei*, *Fusarium moniliformae*, *Gliocladium deliquescens*, *G. roseum*, *Humicola fuscoatra*, *H. nigrescens*, *Paecilomyces variotii*, *Penicillium chrysogenum*, *P. oxalicum*, *P. purpurogenum* and *P. rugulosum* accounted for less than 1% of the total CFUs isolated by the culture plate method.

Similarly, according to the rotorod measurements, the fungal spores contributing less than 1% to the annual total airspora were *Beltrania*, *Botryosphaeria*, *Botrytis*, *Cordana*, *Corynespora*, *Curvularia eragrostidis*, *Dictyoarthrinium*, *Diplodia*, *Haplosporella*, *Helminthosporium*, *Heterosporium*, *Memmoniella echinata*, *Monascus*, *Othia*, *Pithomyces*, *Pyricularia oryzae*, *Sordaria*, *Tetraploa aristata*, *Trichothecium* and *Xylaria*.

Taking into account the diurnal periodicity, certain fungal spore types were observed with highest incidence at different hours of the day. *Cladosporium*, *Albugo*, *Dictyoarthrinium*, *Trichocladium* and *Penicillium* were found mostly during morning hours, *Nigrospora*, *Cordana* and *Drechslera* during noon hours and *Sporormia*, *Sordaria*, *Teichospora*, *Macrophoma*, *Botrytis*, *Corynespora*, *Dendryphion*, *Myrothecium* and *Trichothecium* were recorded in higher amounts during evening hours.

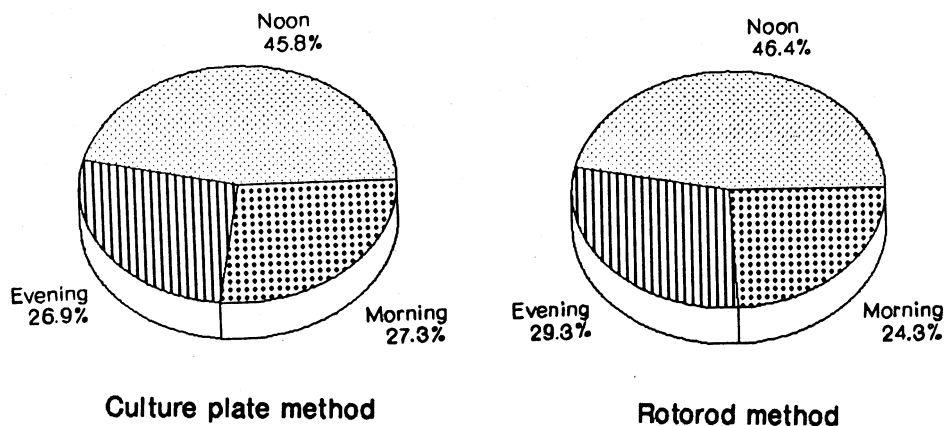


Fig. 3. Diurnal variation in spore concentration.

4. Discussion

Thanks to the present study the existence of a variety of airborne fungal spores in the chloralkali factory under investigation was proven. The number of fungal species isolated by the culture plate method were relatively higher than the fungal spore types trapped with the rotorod. Most of the fungal spores recorded by the rotorod belonged to Deuteromycotina, followed by spore types belonging to Ascomycotina; a few spores were assigned to mucorales. Similar results have been achieved around the world by Larsson (1981), Rubulis (1984), Rai and Singh (1988), Palmas and Cosentino (1990) and Halwagy (1994).

The seasonal impact on airborne fungal spore concentration is quite evident from Fig. 1. A remarkable variation was observed in the monthly concentration of fungal spores. The maximum load of airspora was observed in January and November and the minimum June and July. These variations might be due to the fluctuation in temperature, relative humidity and rainfall. The lowest concentration recorded in June and July coincided to heavy rainfalls whereas the highest contribution assessed in January and November corresponded to low rainfall, moderate temperature and moderate humidity (Srivastava and Shukla, 1990; Nayak and Behera, 1996). Tilak (1989) and Verma (1990) also reported that moderate temperature, moderate humidity and mild rainfall favour the fungal sporulation, whereas extreme temperatures give less support to the process and heavy rainfall washes out spores from the atmosphere.

The present data revealed a marked seasonal fluctuation in the total airspora m^{-3} and spore types over the whole study period (Fig. 1). Fungal spore counts were usually higher in winter, but low counts of spores in summer and even lower ones in the rainy season were reported by culture plate method; instead, with the rotorod, low spore counts were recorded in the rainy

season and lower ones in summer. Similar observations, with more fungal spores in winter (Nayak and Behera, 1996) and more mould spores in autumn and spring (Palmas and Cosentino, 1990) have been recorded earlier, but no investigations have ever reported lower spore concentration in June and July. However, the present study reported a general trend in variation of airborne spore concentrations already found in tropical environments (Talde and Gaikward, 1981; Maribhat and Rajasab, 1991).

Cladosporium was the first dominant fungal spore detected with the culture plate method and the fourth dominant one with the rotorod corroborating thus the findings of earlier reports (Sarma and Sarma, 1993; Satheesh et al., 1993). According to the data achieved with the rotorod, *Nigrospora* was the first dominant fungal: spore accounting for 15.78% of the annual total and occurring throughout the year fully in agreement with earlier data coming from the coastal belts (Varma, 1984) but differing from those of other sites (Srivastava and Shukla, 1990; Maribhat and Rajasab, 1991). With the rotorod hyphal fragments were recorded as the second dominant fungal spore comprising 9.73% of the total airspora. According to some authors (Rees, 1964;

Table 1

Total number of fungal types and similarity coefficient values recorded at different times of the day

	Day times		
	Morning	Noon	Night
Morning	64 ^a	—	—
Noon	82.44 ^b /54 ^c	67	—
Night	75.36/52	76.59/54	74

^a Total number of fungal types.

^b Similarity coefficient.

^c Common number of fungal types between two samples.

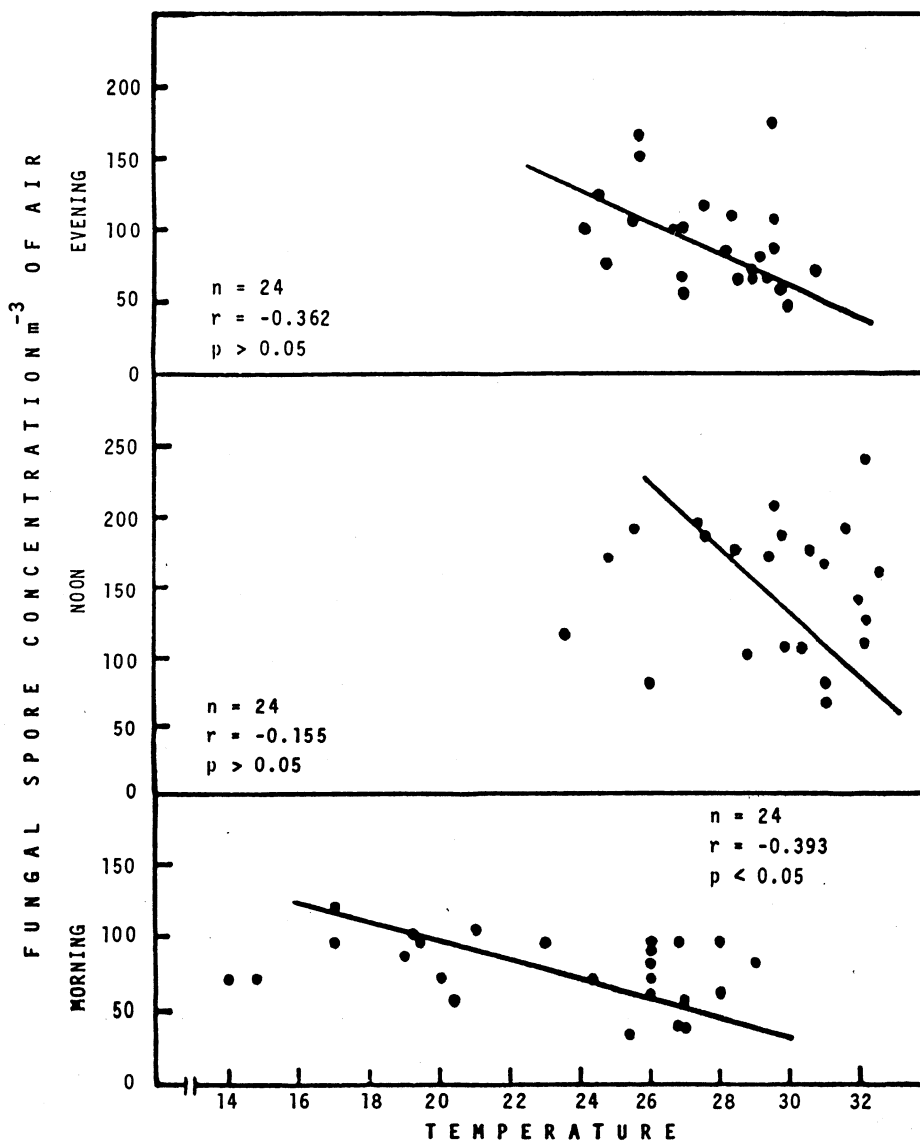


Fig. 4. Pearson's coefficient of correlation of fungal temperature, ' r ' correlation coefficient, ' n ' number of observations and ' P ' probability level.

Ramalingam, 1971; Srivastava and Shukla, 1990) hyphal fragments were ranking first among airspora. In the present study the concentration of hyphal fragments was found to be rather high compared to other places. The high concentration of the fungal hyphae (fragments) in the factory area might be due to the prevailing conditions of the site, that might have caused fungi to be dispersed in the atmosphere in hyphal forms. Kulkarni, (1981) reported in his findings that the effects of relative humidity, temperature and rainfall do not play any significant role in the distribution of fungal hyphae, however, the concentration of hyphal fragments increases in relation to an increase in humidity and wind velocity (Srivastava and Shukla, 1990). The reason for such a high concentration of airborne hyphal fragments might lie in the location of the factory built close to the seaside.

Nayak and Behera (1996) reported that the fluctuation of the atmospheric spore load throughout the year was controlled by the meteorological parameters. When atmospheric fungal spores m⁻³ and meteorological parameters were statistically correlated, the correlation coefficient became negative (Figs. 4 and 5), hence it was confirmed that the independent variables (temperature and humidity) directly regulated the distribution of fungal spores in the air and it was inferred that the increase in temperature and humidity decreases the fungal spores in the air and vice versa. In the present study a negative correlation was found between spore counts and temperature in accordance with the earlier study of Ganguly (1992). It was inferred that with moderate-temperature and relative humidity peak concentration of airborne spores could be recorded.

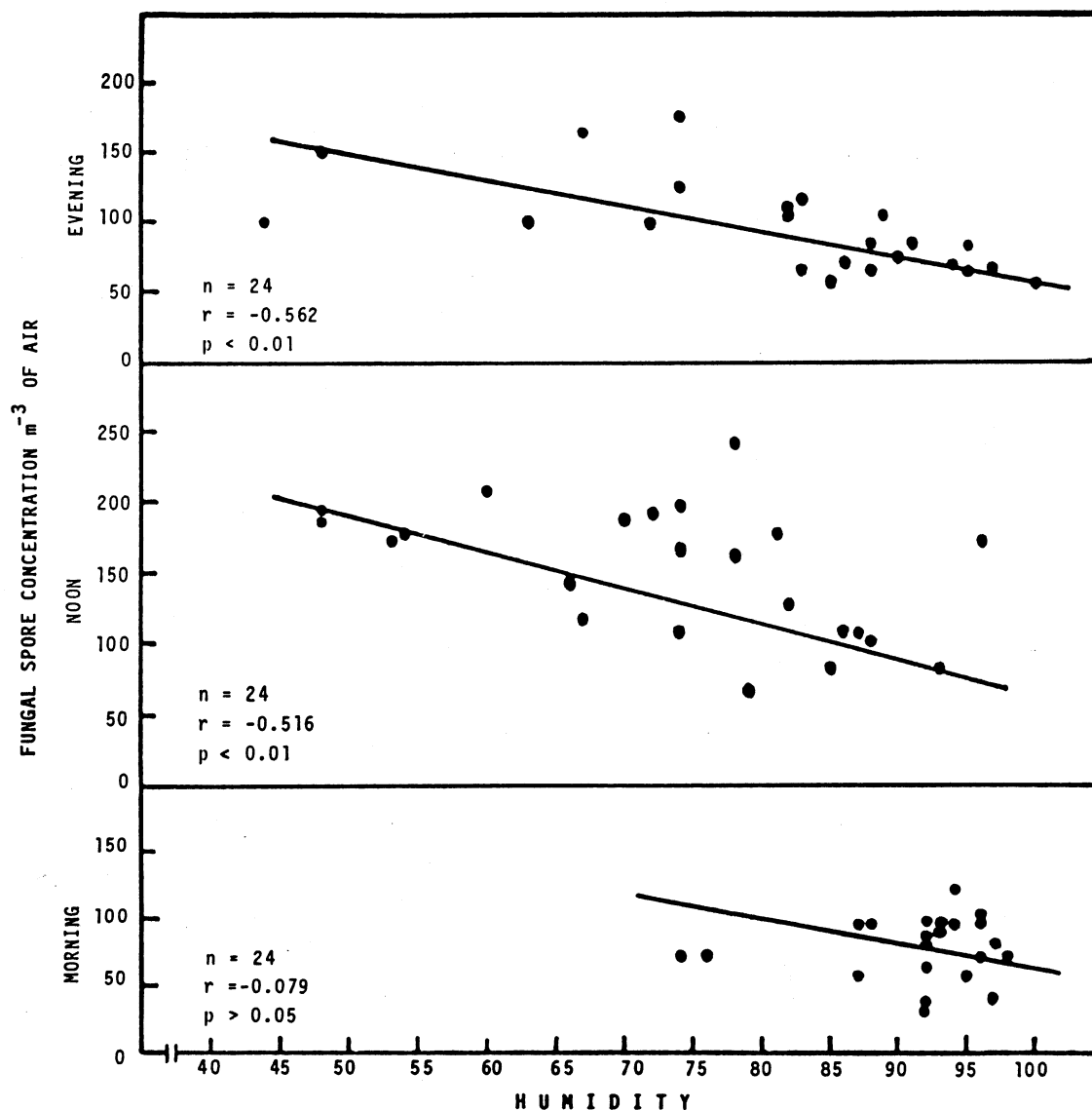


Fig. 5. Pearson's coefficient of correlation of fungal spore concentrations m⁻³ of air versus relative humidity, 'r' correlation coefficient, 'n' number of observations and 'P' probability level.

The dominant ones contributed substantially to the annual total spore and to the day time total (Table 2). Besides, a considerable variety of fungal spores, very low in number, were recorded sporadically over the investigated period. With respect to the responses of individual fungi to season, and to day times, the authors found contradictory and coinciding reports (Khan and Ali, 1984; Kumar, 1984; Janaki Bai et al., 1987; Nayak and Behera, 1996). However, the occurrence of *Alternaria*, aspergilli, penicilli, *Curvularia*, *Drechslera* and a few others was in agreement with the findings of some of the authors (Infante et al., 1987; Jurado et al., 1990).

The study monitored a large number of saprophytes, some plant pathogens and few aeroallergens in the atmosphere of the factory area. The dominant sapro-

phytes were *Cladosporium*, *Pestalotia*, *Curvularia*, *Lasiodiplodia*, *Nigrospora*, *Chaetomium*, *Epicoccum*, *Periconia*, *Memmoniella* and a few others. Plant pathogens like *Alternaria*, *Drechslera*, *Helminthosporium*, *Fusarium*, *Pyricularia*, *Colletotrichum* (Nanda et al., 1996) were recorded at different concentrations. *Alternaria*, *Drechslera* and *Fusarium* were the dominant plant pathogenic fungi accounting for more than 13% of the total airspora and their occurrence was mostly influenced by the vegetation of the site. Aspergilli and penicilli reported to be aeroallergens (Singh and Singh, 1994) and other mould fungi viz., *Mucor*, *Paecilomyces* and *Gliocladium* were isolated in good numbers.

A. flavus, causing 'aflatoxicosis' in birds by producing aflatoxin (Verma and Bhandari, 1992), *A. fumigatus*, the main agent causing 'aspergillosis', commonly

Table 2

Annual percentage abundance of fungal types contributed above 1% in the atmosphere of the factory area during 1993

Fungi	Day times							
	Morning		Noon		Evening		Average %	
	CP	RM	CP	RM	CP	RM	CP	RM
<i>Albugo</i>	—	7.04	—	0.57	—	1.34	—	2.37
<i>Alternaria alternata</i>	2.88	4.88	4.16	5.10	2.70	3.81	3.42	4.67
<i>A. Padwickii</i>	—	3.25	—	1.13	—	0.89	—	1.58
<i>Aureobasidium pullulans</i>	1.22	—	0.59	—	1.69	—	1.05	—
<i>Aspergillus awamori</i>	2.32	—	2.18	—	4.84	—	2.93	—
<i>A. niger</i>	6.53	—	1.98	—	4.84	—	3.99	—
<i>Chaetomium globosum</i>	—	4.88	—	3.40	—	2.91	—	3.62
<i>Cladosporium</i>	—	4.06	—	5.38	—	12.78	—	7.23
<i>C. cladosporioides</i>	17.49	—	18.68	—	21.93	—	9.28	—
<i>C. colocasiae</i>	6.75	—	4.82	—	4.16	—	5.17	—
<i>C. herbarum</i>	5.20	—	7.79	—	3.48	—	5.93	—
<i>C. oxysporum</i>	4.43	—	0.26	—	1.12	—	1.63	—
<i>C. sphaerospermum</i>	4.87	—	0.00	—	0.45	—	1.45	—
<i>Colletotrichum</i>	—	0.00	—	2.41	—	0.45	—	1.25
<i>Curvularia andropogonis</i>	1.88	1.35	3.50	1.13	4.16	2.91	3.23	1.71
<i>C. eragrostidis</i>	0.44	—	2.04	—	1.57	—	1.48	—
<i>C. lunata</i>	0.66	3.79	4.16	8.64	5.51	4.48	3.57	6.24
<i>C. pallescens</i>	2.44	—	0.73	—	1.69	—	1.45	—
<i>Drechslera australiensis</i>	2.32	8.40	4.55	5.24	5.51	2.24	4.20	5.13
<i>D. rostrata</i>	0.55	1.62	2.11	1.42	2.70	3.48	1.84	2.10
<i>Epicoccum</i>	—	3.52	—	2.69	—	3.81	—	3.22
<i>Fusarium oxysporum</i>	3.21	3.52	4.22	1.98	3.82	2.91	3.84	2.63
<i>Gray sterile mycelia</i>	10.52	—	19.40	—	7.98	—	13.91	—
<i>Hyphal fragments</i>	—	11.11	—	7.93	—	11.43	—	9.73
<i>Lasioidiplodia sp.</i>	2.99	0.27	2.77	3.96	3.37	2.46	2.99	2.63
<i>Leptosphaeria</i>	—	0.00	—	1.84	—	1.79	—	1.38
<i>Nigrospora sphaerica</i>	—	15.17	—	18.98	—	11.21	—	15.78
<i>Periconia</i>	—	5.96	—	4.81	—	3.36	—	4.67
<i>Pestalotia sp.</i>	1.55	3.79	0.99	1.98	1.91	1.34	1.39	2.33
Unidentified spores	—	6.23	—	9.49	—	7.85	—	8.22
<i>Thielavia terricola</i>	—	0.54	—	2.41	—	0.67	—	1.45
White sterile mycelia	7.75	—	3.17	—	4.05	—	4.66	—
Total number of fungi	42	30	43	41	45	40	57	51

CP, culture plate method; RM, rotorod method.

known as breeder's pneumonia in poultry (Nayak, 1994), *A. niger* and *A. awamori* were recorded abundantly.

Peak concentration and the widest variety of fungal spores were recorded at noon followed by morning and evening values. This difference in the total number of spores might be due to the temperature at noon, which, enhancing wind speed, might have been causative in dispersing higher amounts of spores from substrata thus giving rise to higher spore concentration in the atmosphere (Kumar, 1984). Early in the morning, spore dispersal was hindered by dew drops. Moreover wind turbulence was much weaker than at noon (Pedgley, 1991). Both events might have been the cause of the low concentration during morning hours (Srivastava et al., 1990). A low spore profile in the evening might be due to the lower humidity, non

sporulation in the second half of the day and to the spore settling during daytime (Nayak, 1994). With the culture plate method the maximum occurrence of *Cladosporium* spores was recorded in winter, specifically during morning hours rather than at noon and in the evening. *Cladosporia* are seasonal fungi abundantly found during the winter. In addition, the dispersal of these spores (Ingold, 1971), recorded in higher amounts in culture plates, is enhanced by the small water particles (mist) found early in the morning.

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