Mycopathologia **149:** 35–45, 2000.
© 2001 *Kluwer Academic Publishers. Printed in the Netherlands.*

Incidence of allergenically significant fungal aerosol in a rural bakery of West Bengal, India

Atin Adhikari, Moon Moon Sen, Swati Gupta-Bhattacharya & Sunirmal Chanda
Division of Palynology and Environmental Biology, Department of Botany, Bose Institute, Calcutta - 700 009, India

Abstract

The frequency of fungal spores in the air of three different sections of a rural bakery was analyzed using a Burkard personal slide sampler and Andersen two stage viable sampler. In average concentration of spores (No./m^3) was 228–26770/m^3 and concentration of viable colony forming units (CFU/m^3) was 65-2061 CFU/m^3. Dominant fungus species both culturable and nonculturable, were species of *Aspergillus* and *Penicillium*, *Cladosporium* sp., *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Cladosporium cladosporioides*, *Penicillium citrinum* and *Alternaria alternata.* Seasonal variations in the spore concentrations were clearly observed in case of some fungi. Total culturable mould concentration of different bakery sections sometimes exceeded the acceptable limit for a healthy indoor environment. Antigenic extracts prepared from some dominant culturable fungi showed high level of allergenicity in skin prick tests indicating that they could be responsible for allergic respiratory dysfunction of bakery workers.

Key words: bio-aerosol, fungal spore, bakery, allergy, aerobiology

Introduction

Fungal spores are important components of bio-aerosol in both indoor and outdoor environments. They may be either a single organism unit or cluster of several organisms. It has been demonstrated that fungal spores are present in high concentration in the air of work places[1–3] including a bakery [4, 5]. In the relevant work places, it is assumed that airborne fungal spores could cause occupational diseases such as respiratory tract allergy, allergic rhinitis, bronchial asthma, other lung diseases and organic dust toxic syndromes [6, 7]. Bakeries are important food industries in India where large number of people are occupationally involved. In rural areas of India. including West Bengal, most bakeries are fitted with old fire oven system for baking instead of incubation chambers resulting in higher temperatures in the baking place and other sections. High temperatures and storage of raw organic materials (e.g., flour, unbaked breads, pastries, etc.) result in inadequate sanitary arrangements in which a wide range of thermophiles and other fungi can grow. Although there is information on airspora of modernized city bakeries of India [4], information on the fungal aerosols of rural bakeries using old baking systems and their allergic potentiality is not adequately known. The objectives of the present study are to record the culturable and nonculturable airborne fungi in a rural bakery using modern aerobiological sampling techniques and to study the allergenicity of dominant culturable fungi on the people who visit or are occupationally involved in the bakeries.

Materials and methods

Description of the selected bakery and associated characteristics

The rural bakery in which this investigation was conducted was situated at Banyapara in Howrah District of West Bengal (about 20 km. south-west of Calcutta). Although the bakery was situated in a rural place its production rate was very high. About 200 people were

working in different shifts and most of them were involved with processing and baking. Air sampling was done from the following sections, i.e., baking section, packing section and office.

The baking section had three large woodfire ovens, large bowls for mixing flours with water and other ingredients, stacks of trays with baked and unbaked goods. Temperature was higher than in the other sections. In the packing section baked pieces of cakes, breads, biscuits and pastries were packed. The place was mainly for packing, however the ingredients of pastries were processed on one side. The office was relatively clean and well ventilated, somewhat similar to other indoor home environments.

Sampling procedures

The samples were collected from October, 1996 to September, 1997 using a Burkard Personal Slide Sampler (Burkard Manufacturing Co. Ltd., Hertfordshire WD) 3 IP, U.K.) and Andersen Two Stage Viable Sampler [8] (Graseby Andersen, Smyrna, 300082 - 5211, U.S.A.), three times a month, at 10 day intervals between 9.30 a.m. and 12.30 noon. The Burkard Personal Slide Sampler was used to trap both culturable and nonculturable spores, whereas Andersen two Stage Viable Sampler was used to trap 95% or more of the viable airborne fungal spores that were above 0.8μ size. Burkard Personal Slide Sampler samples air at a rate of 10 l per minute and it was operated for 5 min. However, Andersen Two stage Viable Sampler moves air at a rate of 28.3 l/min and was operated for 3 min. During sampling runs both of the samplers were placed at 1 m height. The sampling hours, sampling times and sampling height was selected after preliminary studies for the standardization of the techniques.

The Burkard Personal Slide Sampler moves air through a rectangular orifice at the top and particles are impacted on a glycerine jelly coated microslide in the form of a band. The exposed slides were then mounted and examined microscopically to identify and count fungal spore types. The results was then expressed as spores/m^3 air.

Andersen Two Stage viable Sampler moves air through the orifice at the top and impinges successively onto two Petri plates containing 27 ml of Sabourauds Rose Bengal Agar. After exposure, the Petri plates were incubated in a B.O.D. incubator (27 ± 2 °C) for 2/3 days for growth of fungal colonies for identification. The number of colonies recorded was then expressed as number of colony forming units per cubic meter (CFU/m^3) of air sampled.

Recording of meteorological parameters

Temperature (°C) and relative humidity (%) were recorded in each place during sampling time using a Wet & Dry Bulb Hygrometer (G.H. Zeal Ltd., London, U.K.). The rainfall data was from the meteorological office at Alipore.

Skin-prick test [9] to study the degree of allergenicity of fungi

From selected dominant culturable moulds antigenic extracts were prepared. Mass cultures of fungi were prepared in sterile Sabouraud's liquid broth medium in conical flasks. Flasks were incubated at 27 ± 2 °C for about 4 weeks, until sporulation occurred. The medium was then decanted, mycelial pellets rinsed with distilled water and kept overnight in absolute ethyl alcohol. The pellet was then dried, powdered in a grinder and defatted in diethyl ether. Antigenic extracts were then prepared from mycelial powders in 10% (1:10 w/v) concentration in 0.05 M ammonium bicarbonate buffer (pH 8.1). The extraction was done for 24 h with continuous stirring using a magnetic stirrer at 4 °C after which the suspension was centrifuged at 21,000 rpm for 30 min at 4 °C. The supernatant was dialysed against distilled water with Visking tubing (mol. wt. cut off limit 10,000) overnight, followed by centrifugation, filtered through a Milipore filter (0.22 μm), stored at -20 °C in sterile vials until used for the skin-prick test.

The skin-prick test was performed on the ventral side of the forearm. 0.01 ml to 0.02 ml antigens of 1:50 dilution placed in parallel array to the axis of each forearm cleaned with sterile alcohol at a distance of two centimeters. The sites were then pricked with 26 No. hypodermic needle. To confirm the state of reactivity of skin, histamine di phosphate (1 mg/ml) and buffered saline dilutant were used as positive and negative control respectively. The result were recorded after 20 min and allergenic reaction were graded into '+', '++', '+++', '++++' and '$-$' on the basis of intensity of reactions, erythema or wheal formation.

Results

In average the spores of Deuteromycotina were the largest contributors of the total airborne fungal spores.

Spores of 36 fungal genera (of which 21 belonging to Deuteromycotina) were recorded from slides exposed in the Burkard personal slide sampler and viable spores of 34 fungal species belonging to 17 genera (of which 10 belonging to Deuteromycotina) were recorded from plates exposed in the Andersen two stage viable sampler from three different sections of the bakery. The spores of '*Aspergillus/Penicillium* group' dominated in all the three sections. Other dominant spore types were *Cladosporium*, *Alternaria*, *Nigrospora*, *Periconia*, *Drechslera*, and *Torula*. Some ascospores and basidiospores in a higher concentration remained unidentified. Dominant culturable moulds originated from *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Cladosporium cladosporioides*, *Penicillium citrinum* and *Alternaria alternata*.

In all the sections total spore concentration varied between 228-26770/m^3 and the concentration of culturable moulds varied between 65-2061/m^3 (Figures 1 and 2) where counts from all bakery section were considered. Average maximum spore and CFU concentration was observed during the months of August and September and later in February and March, i.e., in late monsoon season and late winter.

A detailed account on the monthly variations of data from the two sampling techniques in three sections of the bakery are presented.

In the baking section when sampling was done with a Burkard personal slide sampler, species of *Aspergillus* and *Penicillium* were recorded in a rather high concentration (25490/m^3) during September. Other common fungus genera were *Periconia*, *Cladosporium* and *Drechslera*. Monthly variations of the concentration of 16 common fungus genera are presented in Table 1. Beside these 16 *Choanephora*, *Botryodiploidea*, *Thermomyces*, *Memnoniella*, *Spegazzinia*, *Trichothecium* and *Leptosphaeria* were recorded sporadically in a higher concentration than other rare types.

In the baking section when sampling was done with the Andersen two stage viable sampler maximum CFU concentration was *Aspergillus flavus* (985 CFU/m^3) during September followed by *Cladosporium cladosporioides* (579 CFU/m^3) during February and *Aspergillus niger* (311 CFU/m^3) during July.

Monthly variations of the concentration of 16 dominant moulds are presented in the Table 4. *Aspergillus glaucus*, *Aspergillus nidulans*, *Aspergillus candidus*, *Scopulariopsis brevicaulis*, *Paecilonzyces varioti*, *Trichoderma harzianum*, *Cephalosprium* sp. and *Chaetomium globosum* showed relatively higher CFU concentration than the other rare types.

When sampling was done with the Burkard personal slide sampler, the concentration of species of *Aspergillus* and *Penicillium* was much lower in packing section during September (1833/m^3). However, during April there was a higher concentration (2827/m^3) than the baking section. Other genera were *Cladosporium*, *Alternaria*, *Curvularia*, *Nigrospora*, *Periconia*, *Corynespora*, *Ganoderma* and also smut spores. Monthly variations in the concentration of 16 genera recorded in this section are presented in Table 2. *Botryodiplodia*, *Cylindrocarpon*, *Deightoniella*, *Heterosporium*, *Memnoniella*, *Phaeotrichoconis*, *Spegazzinia*, *Tetraploa*, *Tetracoccosporium* and *Leptosphaeria* were sporadically recorded in a higher concentration than the other rare genera. When sampling was done with the Andersen two stage viable sampler in the packing section, maximum CFU concentration was by *Aspergillus flavus* (1577 CFU/m^3) during September followed by *Aspergillus japonicus* (493 CFU/m^3) during July and *Penicillium restrictum* (485 CFU/m^3) during August. Monthly variations of the concentration of 16 dominant culturable moulds recorded in this place are presented in Table 5. Other than these common types, *Aspergillus candidus*, *Candida albicans*, Yeasts, *Monilia sitophila*, *Gliocladium roseum*, *Paecilomyces lilacinus*, *Scopulariopsis brevicaulis*, *Stachybotrys* sp., *Trichoderma harzianum*, *Cephalosporium* sp., *Chaetomium globossum* and *Absidia corymbifera* showed higher CFU concentration.

When sampling was done in the office section with the Burkard personal slide sampler species of Aspergillus and Penicillium was recorded in a much lower concentration (786/m^3 during September) than in the baking and packing sections. Most of the other common genera recorded in baking and packing sections were also recorded in the office section in a lower concentration (Table 3). Smut spores were recorded here in high concentration. *Pithomyces*, *Dyctiosporium* and *Rosellinia* were recorded sporadically in a slightly higher concentration than the other spore types.

When sampling was done with the Andersen two stage viable sampler in the office section, the highest CFU concentration was by *Cladosporium cladosporioides* (1049 CFU/m^3) during February, by *Aspergillus fumigatus* (624 CFU/m^3) during February and by *Aspergillus flavus* (548 CFU/m^3) during June. Monthly variations of the concentration of 16

Table 1. Monthly average spore concentration (No. of spores per cubic meter of air) recorded with Burkard Personal Slide Sampler in the baking section

Fungal spore types	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.
Alternaria				7.3	50.0	20.0	6.7	86.7	20.0			
Aspergilli/Penicilli	574	330	174	870	2090	173	147	6713	387	1173	187	25490
Cladosporium	244	315	26	1056	410	660	527	167	200	20		
Choanephora					6.7	6.7		20.0				
Corynespora	14.7				20.0		6.7	6.7				
Curvularia	29.3	11.0		3.7		6.7		20.0	13.3	6.7	13.3	20.0
Drechslera	29.7	37.0	14.7	37.0	20.0	33.3	20.0	60.0	100		13.3	240
Epicoccum				3.7		3.7	6.7					
Nigrospora	52	48	29.3	22.0	10.0	40.0	153	120	26.7	180		70.0
Periconia	7.33		3.7	100	90	33.3	93.3	46.7		147		
Torula	11.0	11.0	7.3	3.7	20.0		13.3	20.0		10.01		
Leptosphaeria								20.0		53.3	80.0	
Ascospores		14.7		70.3	10.0	6.7	6.7	20.0	53.3	313	147	610
Ganoderma	14.7	25.7						20.0	60.0	127	127	200
Smut spores	14.7	14.7	3.7	18.3	10.0		40.0		13.3			
Unidentified basidiospores	18.3	29.3		7.3	10.0	26.7	86.7	6.7	86.7	13.3	93.3	70.0

Table 2. Monthly average spore concentration (No. of spores per cubic meter of air) recorded with Burkard Personal Slide Sampler in the packing section

Fungal spore types	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.
Alternaria						13.3	33.3	33.3	13.3	2033		20.0
Aspergilli/Penicilli	245	459	78.0	107	260	540	2827	46.7	220		490	1833
Cladosporium	215	881	839	219	440	1620	1387	33.3	93.3	46.7	160	
Choanephora												
Corynespora	7.3	18.3			10.0		46.7		6.7			
Curvularia	14.7	22.0	14.7	11.0	10.0		26.7	60.0	6.7		30.0	
Drechslera	33.0	25.7	22.0	18.3	20.0	40.0	33.3	13.3	66.7	40.0		20.0
Epicoccum					20.0	33.3	6.7	6.7				6.7
Nigrospora	29.3	29.3	27.5	25.5	50.0	40.0	100	93.3	80.0	93.3	40.0	106
Periconia					80.0	53.3	53.3	46.7	60.0	26.7		
Torula	11.0	11.0	5.5								30.0	33.0
Leptosphaeria								13.3	33.3	66.7		
Ascospores	18.3	40.3	11.0	7.3	150	20.0	106			166	70	33.3
Ganoderma	33.0	3.7	16.5						60.0	53.3	80.0	73.3
Smut spores	7.3	3.7	11.0	3.7	30.0	427				6.7		13.3
Unidentified basidiospores	22.0	11.0	11.0	7.3	30.0	6.6	33.3	20.0	20.0	60.0	133	

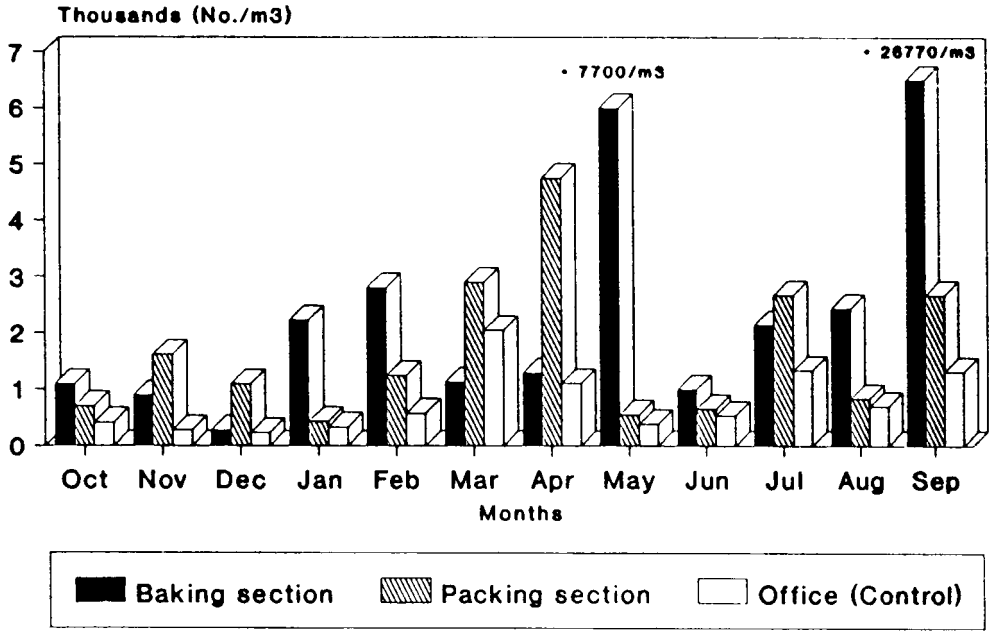

Figure 1. Monthly averages of total spore concentration (No./m^3) in different sections of the bakery.

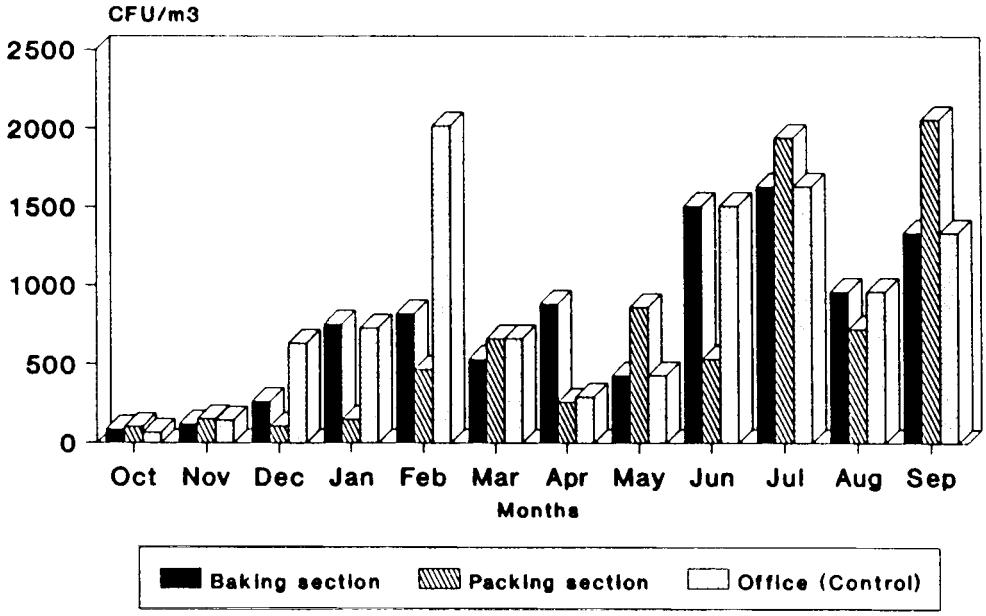

Figure 2. Monthly averages of total viable spore concentration (CFU/m^3) in different sections of the bakery.

dominant fungus genera recorded in the office section are presented in the Table 6. *Aspergillus parasiticus, Aspergillus candidus, Aspergillus terreus, Aspergilliis clavatus, Drechslera hawaiiensis, Drechslera coisis, Trichoderma harzianum, Candida albicans, Paecilomyces lilacinus, Paecilomyces varioti, Cephalosporium* sp. and *Absidia corymbifera* were recorded in a higher concentration.

Record of meteorological parameters

Temperature (°C) and relative humidity (%) were recorded during each sampling. The data on average

Table 3. Monthly average spore concentration (No. of spores per cubic meter of air) recorded with Burkard Personal Slide Sampler in the control sampling spot (office)

Fungal colony types	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.
Alternaria	7.3	14.7	33.0	14.7	40	20.0	20.0		6.7			
Aspergilli/Penicilli	326	126	22.3	37	387	127	233	113	560	86.7	786	
Cladosporium		37.0	29.7	141	180	93.3			280		80.0	
Choanephora					10.0		13.3					
Corynespora		18.3					13.3				6.7	
Curvularia		22.01				6.7	33.3	60.0	6.7		40.0	6.7
Drechslera	11.0	14.7	22.0	25.7	40.0	13.3	40.0	40.0	46.7	40.0	26.7	53.3
Epicoccum					20.0		20.0					
Nigrospora	11.0	18.3	51.7	44.3	90.0	60.0	120	33.3	40.0		33.3	46.7
Periconia				11.0	100	40.0	113					
Torula										20.0	86.7	13.3
Leptosphaeria										10.0	6.7	
Ascospores		18.3	14.7	11.0	10.0	46.7	147			280	120	133
Ganoderma	7.3	14.6	11.0	11.0	10.0				26.7	90.0	60.0	126
Smut spores						1160	253					6.7
Unidentified basidiospores	22.0	7.3	11.0	11.0	10.0	20.0	27.0		13.3		113	13.3

Table 4. Monthly average CFU concentration (No. of colony forming units per cubic meter of air) recorded with Andersen Sampler in the baking section

Fungal colony types	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.
Alternaria alternata	5.3	5.3	8.0	17.0	6.0	55.3	67.0	8.0	47.0	146		
Aspergillus niger	19.6	28.6	13.3	17.0	6.0	23.6	86.6	110	43.3	311	18.0	27.6
Aspergillus flavus	14.6	21.0	10.6	254	6.0	59.0	11.6	169	167	114	41.0	985
Aspergillus fumigatus	6.3	190	8.0	5.3		35.3	80		31.6	8.0		
Aspergillus japonicus	4.0	5.3						35.3	701	599		
Aspergillus ochraceous	6.6	4.0	6.6	4.0	17.5					14.0		
Cladosporium cladosporioides		6.6	154	342	580							
Cladosporium oxysporum				2.6	17.5	35.3						
Fusarium roseum			8.01	117		8.0		4.0	12.01		19.6	
Penicillium citrinum	11.7		9.3	8.0	23.5	23.6	70.6	43.3	4.0	133	119	
Penicilium chrysogenum							19.61		12.0	59.0		
Absedia blakesliana	4.0	4.0								8.0	18.0	
Mucor raceinosus		1.3	1.3	21.0			8.0					
Rhizopus nigricans	5.3	4.0	2.6	17.0		23.6	19.6					
Rhizopus stolonifer			1.3				8.0	4.0		98.6		
Syncephalastrum raceinosum			1.3				8.0	4.0		98.6		

monthly rainfall was collected from the nearest meteorological office. Average monthly temperature, relative humidity and rainfall have been presented in Table 7.

Results of skin-prick test

Results showing higher frequencies of positive reactions in case of nine culturable fungi are presented in Table 8. Four grades of reactions were observed: + (2–3 mm), ++ (4–5 mm), +++ (6–8 mm) and ++++

Table 5. Monthly average CFU concentration (No. of colony forming units per cubic meter of air) recorded with Andersen Sampler in the packing section

Fungal colony types	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.
Alternaria alternata		4.0	5.3	4.0			35.6		4.0	201		4.0
Aspergillus niger	19.6	22.3	10.6	13.3	12.0	23.6	67.0	420	83.0	55.0	23.6	
Aspergillus flavus	18.3	19.6	18.0	14.6	29.5	170	47.3	136	83.0	47.3	47.0	1577
Aspergillus fumigatus	1.3	28.6	9.0	5.3	12.0				39.3	493		
Aspergillus japonicus	6.6	8.0	4.0					59.0	39.3	493		
Aspergillus ochraceous							8.0	6.0	4.0	4.0		
Cladosporium cladosporioides		9.3	18.3	47.0	65.0	173	4.0					
Cladosporium oxysporum						5.3						
Fusarium roseum					18.0		8.0		23.6			
Penicillium citrinum			11.6	8.0	83.0	98.6		35.5		23.7	4.0	4.0
Penicilium chrysogenum						23.6	11.6		4.0	303	74.6	
Absedia blakesliana	6.6	2.6				8.0			8.0		23.6	
Mucor racemosus			4.0	4.0	17.5			6.0	4.0			
Rhizopus nigricans	1.3	14.3	2.6	4.0	12.0	35.3	8.0	6.0	8.0			
Rhizopus stolonifer		1.3			12.0	8.0						
Syncephalastrum racemosum			1.3		12.0	39.3				8.0	4.0	

Table 6. Monthly average CFU concentration (No. of colony forming units per cubic meter of air) recorded with Andersen Sampler in the control sampling spot (office)

Fungal colony types	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.
Alternaria alternata						39.3		31.7	122	35.3		
Aspergillus niger	9.3	13.3	1.3	28.7	49.7		35.7	20.5	177	23.7	57.0	27.7
Aspergillus flavus	25.0	5.3	14.3	27.7	75.0	39.3	83.0		548	110	67.3	67.0
Aspergillus fumigatus		55.0	282	104	624	11.7	11.7	55.3	63.3	23.7	39.3	710
Aspergillus japonicus			9.0	11.7	15.7	8.0	4.0	27.3	39.3	19.7		19.7
Aspergillus ochraceous				4.0	26.3	15.7		8.0		35.3	51.31	
Cladosporium cladosporioides		23.3	204	353	1049	248	8.0					
Cladosporium oxysporum				55.0	11.7	27.7						
Fusarium roseum					12.0	22.3	4.0	19.7	12.0	15.7	35.3	59.0
Penicillium citrinum	9.0	70.7	92.7	77.3	146	15.7		4.0	15.7			
Penicilium chrysogenum									8.0			
Absedia blakesliana	1.3											
Mucor racemosus				8.0	9.3							4.0
Rhizopus nigricans	4.0	4.0	2.7	9.3	8.0	23.7		4.0	4.0	8.0		
Rhizopus stolonifer	2.6			2.6	13.0	11.7	23.7					
Syncephalastrum racemosum	1.3	1.3					12.0	8.0				

(>8 mm.) according to the diameter of wheal. Most of the patients showed + (2–3 mm) reactions.

Discussion

This is now well established that fungal spores are involved with respiratory allergic diseases, lung disorders and related health hazards[10–13]. In occupational environments, including bakeries workers may be exposed to a high concentration of airborne fungal spores. Species that produce mycotoxins may contaminate bakery products and may cause other health hazards. Aeromycoflora of some indoor environments in Calcutta and other places [14, 15, 2–4] have been

Table 7. Average temperature and relative humidity recorded in three sections of the bakery and average monthly rainfall of the locality

Months	Temp. (°C)			R.H. (%)			Rainfall (mm)
	BS	PS	OF	BS	PS	OF	
Oct.	31.6	31.3	30.2	65	59	61	5.2
Nov.	30.4	30.0	29.2	79	74	63	0.0
Dec.	29.1	26.7	26.4	45	77	47	0.0
Jan.	28.6	27.4	25.3	52	62	41	2.3
Feb.	28.2	28.0	27.5	59	50	62	0.6
Mar.	31.6	30.5	30.2	56	59	67	0.1
Apr.	33.6	32.1	31.3	57	61	58	4.2
May	35.6	35.2	34.1	68	61	57	5.4
June	35.4	35.3	34.1	77	61	89	3.1
July	32.4	30.2	30.0	84	68	80	19.2
Aug.	33.2	32.1	29.7	66	76	84	15.3
Sep.	33.5	27.7	27.0	85	76	87	17.1

Table 8. Results of skin prick test showing different grades of positive and negative reactions against antigenic extracts of nine dominant airborne culturable fungi

Name of fungal antigens and total number of patients (n) against each	No of patients and their percentage showing four grades of positive and negative SPT sensitivity				
	-ve	+	++	+++	++++
Aspergillus flavus	30	79	8	0	0
(n = 117)	25.6%	67.5%	6.8%		
Aspergillus japonicus 20	78	10	1	0	
(n = 109)	18.3%	71.5%	9.1%	0.9%	
Alternaria alternata	30	47	18	0	0
(n = 95)	31.5%	49.4%	18.9%		
Aspergillus fumigatus	5	67	19	6	1
(n = 98)	5.1%	68.3%	19.3%	6.1%	1.02%
Aspergillus clavatus	18	60	23	0	0
(101)	17.8%	59.4%	22.7%		
Aspergillus candidas	20	35	4	0	0
(n = 59)	37.03%	64.8%		7.4%	
Cladosporium c1adosporioides	42	59	10	1	0
(n = 112)	37.5%	52.6%	8.9%	0.8%	
Fusarium roseum	6	63	17	4	2
(n=92)	6.5%	68.4%	18.4%	4.3%	2.1%
Rhizopus nigricans	7	60	22	2	1
(n = 92)	7.6%	65.2%	23.9%	2.1%	1.08%

Abbr. SPT = Skin Prick test.

investigated but did not deal with bakeries of rural places. The results of the present investigation supplies useful information in the context of occupational health problems of bakery workers of rural areas in tropical countries. In the present study a wide range of airborne fungal spores have been recorded from the three sections of the bakery.

In the baking section using Burkard personal slide sampler a high spore concentration was recorded probably due to the abundance of raw organic materials. Spore concentrations of species of *Aspergillus* and

Penicillium recorded in this section, 25490/m^3 of species of *Aspergi1lus* and *Penicillium* and 26770/m^3 of total spores was much higher than the numbers recorded by earlier workers in an Indian city bakery where 13770 spores/m^3 was recorded [4]. However, with the Andersen two stage viable sampler the concentration of culturable spores was 1336 CFU/m^3. This may indicate that most of the airborne spores in the baking section lost viability due to higher temperatures in the baking section. The hot environment may be responsible for a reduced sporulation resulting in lower CFU concentration [16]. It may be speculated that non viable spores are less hazardous than the viable spores, otherwise the workers in the baking section could have faced a serious respiratory dysfunction problem. A more comprehensive study on the allergenicity of non viable spores in hot environments would have made new input to this problem.

In the packing section where the average temperature was less than in the baking section the difference between the record of Burkard and Andersen samplers was less than the baking section. During September total spore concentration recorded by the Burkard sampler was 2679/m^3 whereas total CFU concentration recorded by Andersen sampler was 2061 CFU/m^3. In most of the sampling days this difference was maintained.

In the office of the bakery, where temperature and relative humidity was the normal environment, in the months of September, February and June total CFU concentration was higher than total spore concentration recorded by Burkard sampler indicating that most of the spores were viable and that the Andersen sampler is more efficient for trapping airborne spores, if they are viable, than the Burkard personal sampler.

Maximum spore concentration and CFU concentration were recorded in the baking section, followed by packing section and office. Fungal spores and CFUs recorded were qualitatively and quantitatively much higher than in healthy indoor environments, e.g., nearby residential homes (unpublished data). The maximum CFU concentration recorded in the bakery was higher than those in the bakeries of temperate areas such as 200 CFU/m^3 was recorded by Lacey & Crook [17] and 1.2×10^3 CFU/m^3 as recorded by Crook et al. [18] in British bakeries, but less than in the city bakeries of India where Singh & Singh [4] recorded about 5000 CFU/m^3.

Yeasts and *Mucor* sp. were recorded in a higher concentration by earlier workers [19, 20] in bakeries of temperate environments, but in the present study their concentration was very low which could have been due to non-germination of spores caused by higher temperature.

There is no internationally accepted spore and CFU concentration for allergenic health risk. According to Reponen et al. [21] and Gravesen [22] a level of 500 CFU/m^3 may be considered as unacceptable and 3000 CFU/m^3 of *Cladosporium* sp. and 100 CFU/m^3 of *Alternaria* sp. may function as threshold limits for evoking allergic symptoms. In case of mycotoxigenic fungi, for, e.g., *Aspergillus flavus*, *Aspergillus ochraceus*, etc. a lower CFU concentration may be responsible for high health risk because toxins present in them could be carcinogenic or could damage the immune response capacity of the lung to other microorganisms [23]. In the present study during the months of January to April and June to September the CFU concentration in the baking section is higher than 500 CFU/m^3. The same situation was observed in the packing section during March and May to September and in the office during December to March and June to September. This indicated that these sections of the bakery could be hazardous for the health of workers if they were exposed for a long time. *Alternaria alternata* showed higher concentration than 100 CFU/m^3 during July in packing and baking sections and during June in office. These levels could provoke allergic symptoms in workers. Rao et al. [24] has reported that when the total concentration of fungi in an indoor environment exceeds 1000 CFU/m^3 this may be considered as a contaminated indoor environment. Considering this interpretation all three sections of the bakery could be considered as contaminated environments during the late monsoon season.

In West Bengal of India the period between October to February is considered to be Winter, March to June Summer, and July to September as Monsoon season. Seasonal variations of fungal spore concentrations were demonstrated clearly by Aspergillus and Penicillium, *Cladosporium*, *Alternaria*, *Periconia*, *Drechslera*, *Nigrospora*, *Ganoderma*, *Leptosphaeria* and by colonies of *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus japonicus*, *Cladosporium cladosporioides*, *Alternaria alternata*, *Penicillium citrinum* and *Fusarium roseum*.

Temperature and relative humidity play an important role in fungal spore generation, release and dispersal in both indoor and outdoor environments [23, 25–28]. Lacey [23] reported that culturable moulds like *Aspergillus flavus*, *Aspergillus candidus*, *Aspergillus niger*, *Aspergillus ochraceus*, *Aspergillus fu-*

migatus and *Absidia corymbifera* prefer higher temperature for colonization in cereal grains. The higher concentration of CFU of these fungi in the bakery supported this observation.

The skin-prick test results indicate that several moulds are allergenic, e.g., *Aspergillus japonicus*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Fusarium roseum* and *Rhizopus nigricans*.

For decreasing fungal airborne spore populations in the bakery the following control measures could be adopted: (1) Washing of floors with mould inhibitor chemicals like Thimerosol (0.02%), Formaldehyde (0.2–0.5%) or other chlorine bleaches [29]. (2) High moisture concentration could be eliminated by installing good ventilation systems. (3) Some fungicidal paints could be used. (4) During late monsoon season exposure of workers to fungal spores should be reduced by using suitable respiratory protection against spores less than 1 μm diameter[30].

Acknowledgement

The authors acknowledge the receipt of financial support provided by the Ministry of Environment & Forests, Government of India (Sanction No. 42/8/94-RE). They wish to thank to Late Dr. J. Lacey and Dr. H.A. McCartney of Institute of Arable Crop Research, Rothamsted, U.K. for valuable suggestions and to the Manager, Dora Bakery, Banyapara, Howrah, West Bengal for providing access to the different sections of his bakery and other facilities; to Prof. B.P.R. Vittal of the University of Madras for his kind help in rendering identification of fungi and to Dr. I. Roy and Dr. S. Chatterjee of the Allergy Clinic, Institute of Child Health, Calcutta for performing clinical tests. Thanks are also due to Mr. C. Chakroborty for supervising sampling and to Mr. M.M. Nath and Mr. S. Bayen for their active help in sample collection and laboratory works.

References

1. Cosentino S, Palmas F. Assessment of airborne fungal spores in different industrial working environments and their importance as health hazards to workers. Environ Monitor Assess 1991; 16: 127–136.
2. Vittal BPR, Glory A. Airborne fungus spores of a library in India. Grana 1985; 24: 129–132.
3. Singh AB, Singh A. Indoor airborne fungi as important occupational sensitisers in poultry workers. Indoor Built Environ 1996; 5: 138–147.
4. Singh A, Singh AB. Airborne fungi in a bakery and the prevalance of respiratory disfunction among workers. Grana 1994; 33:3 49–358.
5. Simeray J, Mandin D, Chaumont JP. Variations in the distribution of fungal spores in the atmosphere of bakehouses. Impact on the study of allergies. Grana 1995; 34: 269–274.
6. Latgé JP, Paris S. 1991. The fungal spore : reservoir of allergens. In: Cole GT, Hoch HC, eds. The fungal spore and disease initiation in plants and animals. New York: Plenum Press, 1991; 379–401.
7. Lacey J, Dutkiewicz J. Bioaerosols and occupational lung disease. J Aerosol Sc 1994; 25(8): 1371–1404.
8. Andersen AA. New sampler for collection, sizing and enumeration of viable airborne particles. J Bact 1958; 76: 471–484.
9. Stytis DP, Stobo JD, Fudenberg H, Wells JV. Basic and clinical immunology. Lauge Medical Publ Maruzen Asia (Pvt) Ltd, 1982: 409.
10. Salvaggio J, Aukrust L. Mold induced asthma. J Allergy Clin Immunol 1981; 168: 327–346.
11. Horéjsí M, Sach J, Tomsíková A, Med A. A syndrome resembling farmer's lung in workers inhaling spores of Aspergillus and Penicillia moulds. Thorax 1960; 15:212-217.
12. Patterson R, Sommers H, Fink JN. Farmer's lung following inhalation of *A. flavus* spores growing in mouldy corn – A case study. Clin Allergy 1974; 4: 79–86.
13. Lacey J. Aerobiology and Healt : the role of airborne fungal spores in respiratory diseases. In: Hawksworth DL. ed. Frontiers in Mycology, Honorary and General Lectures from the Fourth Int. Mycological Congress, Regensburg, 1990: 157–185.
14. Santra SC, and Chanda S. Airborne fungal flora in indoor environments of the Calcutta metropolis, India. Grana 1989; 28: 141–145.
15. Chakraverty R. The incidence of *Aspergillus parasiticus* in the indoor and outdoor environments of Calcutta, India. Grana 1985; 24:133–135.
16. Levetin E, Horowitz L. A one-year survey of the airborne molds of Tulsa, Oklahoma. I. Outdoor survey. Ann Allergy 1978; 41: 25–27.
17. Lacey J, Crook B. Fungal and actinomycete spores as pollutants of the workplace and occupational allergens. Ann Occup Hyg 1988; 32: 515–533.
18. Crook B, Venables KM, Lacey J, Musk AW, Newman Taylor AJ. Dust exposure and respiratory symptoms in a UK bakery. In Griffiths WD. ed. Aerosols – Their generation, behaviour and application. Aerosol Society, Bristol, 1988: 341–345.
19. Wallerstein G, Bergmann I, Rebohle E, Gemeinhardt H, Thurmer H. Berufliche A temtrakterkrankungen durch Schimmelpilze bei Getreidemullern und Backern. Zeitschrift fur Efkrankungen der Atmungsorgane 1980; 154: 220–233.
20. Dutkiewicz J. Atmospheric microflora as a harmful factor in bakery working environment. Medycyna Wiejska 1983; 9: 85–99.
21. Reponen T, Nevalainen A, Jantunen MJ, Pelica M, Kalliokoski P. Normal range criteria for indoor air bacteria and fungal spores in a subarctic climate. Indoor air 1992; 2: 26–31.
22. Gravesen S. Fungi as a cause of allergic disease. Allergy 1979; 34: 135–154.
23. Lacey J. Indoor aerobiology and health. In: Singh J. ed. Building Mycology. Chapman & Hall, London, 1994: 77–129.
24. Rao CY, Burge HA, Chang JC. Review of quantitative standards and guidelines for fungi in indoor air. J Air Waste Manag Assoc 1996; 46(9): 899–908.

25. Herrero B, Zaldivar P. Effects of meteorological factors on the levels of *Alternaria* and *Cladosporium* spores in the atmosphere of Palencia, 1990–92. Grana 1997; 36: 180–184.
26. Larsen TO, Frisvad JC. Production of volatiles and mycotoxins in conidia of common Penicillia and Aspergilli. In: Samson RA et al., eds. Health implications of fungi in indoor environments. Elsevier, Amsterdam, 1994: 251–279.
27. Sneller MR, Hayes HD, Pinnas JL. Frequency of airborne Alternaria spore in Tucson, Arizona over a 20-year period. Ann. Allergy 1981; 46: 30–33.
28. Sneiler MR. Mould allergy and climate condition. In: Al-Doory Y, Doinson JF. eds. Mould Allergy. Lea & Febiger, Philadelphia, 1984: 244–266.
29. Standard PG, Kaufman L. Safety considerations in handling exoantigen extracts from pathogenic fungi. J Clin Microbiol 1982; 15: 663–667.
30. Lacey J, Nabb S, Webster BT. Retention of actinomyces spores by respirator filters. Ann Occup Hygiene 1982; 25: 351–363.

Address for correspondence: Dr. Swati Gupta-Bhattacharya, Division of Palynolgy & Environmental Biology, Department of Botany, Bose Institute, 93/1, Acharya Prafulla Chandra Road, Calcutta - 700 009, India
Tel.: 91-33-350-6619; Fax: 91-33-350-6790;
E-mail: swati@bosemain.boseinst.ernet.in