

Volumetric assessment of airborne fungi in two sections of a rural indoor dairy cattle shed

Atin Adhikari*, Moon M. Sen¹, Swati Gupta-Bhattacharya, Sunirmal Chanda²

Division of Palynology and Environmental Biology, Department of Botany, Bose Institute, 93/1 Acharya Prafulla Chandra Road, Calcutta 700 009, India

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Abstract

There is increasing concern about the exposure to fungal aerosols in occupational environments and associated respiratory allergic diseases and asthma. A large number of people work in cattle sheds around the world, pulmonary function impairments and higher frequency of respiratory symptoms have been reported in dairy farmers; however, it appears that adequate information on the fungal aerosols from the cattle sheds are largely lacking. Volumetric assessment of airborne culturable and nonculturable fungal spores was performed in two sections of a large rural indoor cattle shed of West Bengal, India for 2 consecutive years. An Andersen Two Stage Viable Sampler was used for sampling culturable fungi and a Burkard Personal Slide Sampler was used to collect the total airborne fungal spores including both the culturable and nonculturable types. A total of 31 spore types and 35 types of viable colony-forming units were recorded. Average concentration range of total fungal spores was 233–2985/m³ and concentration of viable colony-forming units ranged between 165 and 2225 CFU/m³. Burkard Sampler showed higher frequencies of *Aspergilli*/Penicilli, *Cladosporium*, *Alternaria*, and smut spores. Andersen Sampler showed the prevalence of *Aspergillus niger*, *Aspergillus flavus* and *Cladosporium cladosporioides* colonies. Some recorded fungi were earlier reported as allergenic, toxic, and pathogenic for occupational workers as well as cattle population. Higher concentration levels of airborne total and culturable fungal spores were recorded during the months of November through February (winter) and June through September (late summer and rainy season).

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1. Introduction

Fungal spores constitute a significant fraction of airborne bioparticles (Ebner and Haselwandter, 1992; Takahasi, 1997) and they are often 100–1000 times more numerous than other airborne bioparticles like pollen grains (Lacey, 1981; Lehrer et al., 1983). In average, man inhales approximately 10 m³ of air per day (Lynch and Poole, 1979), containing among others a large number of fungal spores, probably the largest constituents of microorganisms in bioaerosol, ranging from 3 to 30 µm in size (Stetzenbach,

1998). Fungal spores of >10 µm sizes are deposited in the nasopharynx and are associated with nasal and ocular symptoms, which is generally called hay fever (Luo, 1991). Spores <10 µm size, especially those of <6 µm size, can be transported to the lower airways and lungs where allergic reactions sometimes manifest as asthma (Pepys, 1965). Higher frequencies of airborne fungal spores were recorded by many workers from occupational environments (Lacey and Crook, 1988; Vittal and Glory, 1985; Singh and Singh, 1996) including a few studies from dairy barns (Hanhela et al., 1995; Kullman et al., 1998).

In rural areas of India and other tropical as well as temperate countries, a large number of people are occupationally involved with different types of cattle sheds. In these sheds, a wide range of fungal growth substrates like moldy livestock foods, moldy hay, bedding of animals and their excreta are present, which could provide a huge airborne fungal spore load making these places unhygienic for the workers. Consistently, more respiratory symptoms and impaired levels of respiratory function among the dairy

* Corresponding author. Present address: Center for Health-Related Aerosol Studies, Department of Environmental Health, University of Cincinnati, 3223 Eden Avenue, PO Box 670056, Cincinnati, OH 45267-0056, USA. Tel.: +1-513-558-0500; fax: +1-513-558-2263.

E-mail address: adhikaa@email.uc.edu (A. Adhikari).

¹ Division of Pediatrics, Allergy, and Immunology, Arkansas Children's Hospital Research Institute, Little Rock, AR 72202, USA.

² Center for Study of Man and Environment, CK-11, Sector-2, Salt Lake City, Calcutta 700 091, India.

farmers were reported by many researchers (Dalphin et al., 1998a,b; Westeel et al., 2000) compared to the control subjects. Westeel et al. (2000) concluded that the respiratory allergy played a role on the impaired respiratory function among the dairy workers and allergic sensitization to some fungi constituted a risk factor for the development of respiratory airflow obstructions. It appears, however, that adequate information on the fungal aerosols from the cattle sheds are largely lacking.

The objective of the present study was the volumetric assessment of both culturable and nonculturable airborne fungal spores in two sections of a large cattle shed, one for buffalos and the other for cows, during 2 successive years using both viable and non-viable volumetric sampling methods.

2. Materials and methods

2.1. The sampling site

The cattle shed selected for this investigation was situated at Katlia in Howrah District [about 20 km south-west of Kolkata (Calcutta)] in the tropical Gangetic belt of West Bengal, India. This cattle shed was one of the largest cattle sheds in the Howrah district. Two sections of the shed were selected for the study: one was designated for cows and the other for buffalos. Two sections were different in terms of several characteristic features, e.g. the ventilation, drainage, sanitary system, and the surroundings. In the buffalo section, the floor was always wet, sanitary and sewage system was not adequate, fecal material and urine of buffalos was always present and the ventilation was poor. Close to the shed were livestock food storage places, large feed preparation bowls and places for cutting straw manually. Musty odor was common in this place, which may be due to fungal or bacterial growth in rotting organic materials. In the cow section, however, there was adequate arrangement for cleaning the floor with better sanitary and sewage systems. Electric fans were there for keeping the place cool and a number of large exhaust fans were fitted for maintaining proper ventilation. This section was also used for milk processing and selling. Both sections were highly humid with a slightly lower temperature than the outdoor.

2.2. Sampling procedures

The samples were collected in 2 successive years from October, 1996 to September, 1998 using a Burkard Personal Slide Sampler (Burkard Manufacturing, Hertfordshire WD3 1PJ, UK) and Andersen Two Stage Viable Sampler (Andersen, 1958; manufactured by Graseby Andersen, Smyrna, 300082-5211, USA), three times a month, at 10-day intervals between 9:30 AM and 10:00 AM round the year. Two samples were collected in every sampling day from the

cattle shed (one sample from each section) and altogether 72 samples were collected.

Burkard Personal Slide Sampler was used for sampling of both culturable and nonculturable fungal spores, whereas Andersen two Stage Viable Sampler was used to collect 95% or more of the airborne viable fungal spores above 0.8 μ sizes. Burkard Sampler sampled air at a rate of 10 l per min and it was operated for 5 min. However, Andersen Sampler sampled air at a rate of 28.3 l/min and it was operated for 3 min. During sampling, both the samplers were placed at 1-m height from the ground level near the breathing zone of the workers. The duration of sampling, sampling time of the day and the height of sampling from ground level were selected after the standardization based on a short pilot study.

The Burkard Sampler collected air through a rectangular orifice at the top and the particles were impacted on a glycerin 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 counts were then converted into the number of spores per cubic meter of air (No/m^3).

The Andersen viable Sampler sucked air through the orifice at the top and impinged successively onto two Petri plates containing 27 ml of Sabourauds Rose Bengal Agar media. After exposure, the Petri plates were incubated in a B.O.D. incubator ($27 \pm 2^\circ\text{C}$) for 2–3 days for proper growth of fungal colonies to render identification. Few fungal colonies required 6–7 days time for growth and maturation. The number of colonies recorded was then expressed as number of colony-forming units per cubic meter of air (CFU/m^3) sampled.

2.3. Recording of meteorological parameters

Temperature ($^\circ\text{C}$) and relative humidity (%) were recorded in each place during sampling time using a Wet and Dry Bulb Hygrometer (G.H. Zeal, London, UK). The rainfall data were obtained from the nearest meteorological office at Alipore.

2.4. Statistical analysis

Paired *t*-test was conducted to study the differences between the two sections' airborne total and culturable fungal spore concentrations. Paired *t*-test was selected because the samples were collected in parallel from the two sections of the same cattle shed, i.e. the data sets were related to each other. Similarly, the differences between the 2 years' data of both total and culturable fungi were also studied by paired *t*-test.

3. Results

Mitosporic fungi showed the largest proportion of the total airborne fungal spores (83.4%) as well as culturable

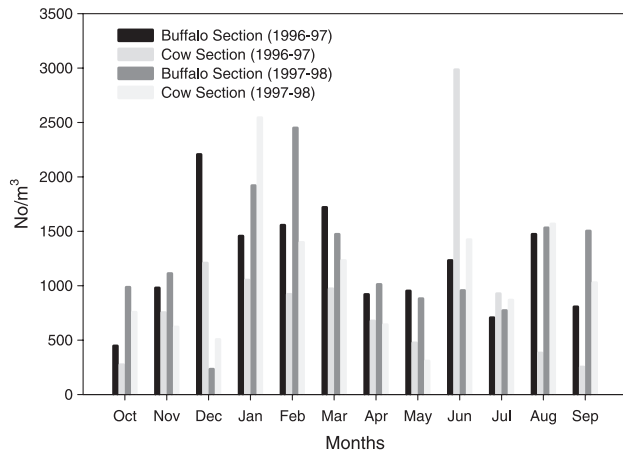


Fig. 1. Monthly averages of total spore concentration (No/m³) in two sections of the cattle shed.

molds (90.6%) from both sections of the cattle shed. A total of 31 spore types and 35 culturable mold types belonging to 17 genera (of which 24 spore types and 12 culturable mold types belonging to mitosporic fungi) were collectively recorded from the two sections of the cattle shed. Average overall concentration range of total fungal spores recorded by the Burkard Sampler was 233–2985/m³ and the concentration of viable fungal colony forming units recorded by the Andersen Sampler ranged between 165 and 2225 CFU/m³ during the whole sampling period in both sections. From both the year's data, it has been observed that the total concentration of spores and CFUs were relatively lower during the months of April–May and July, i.e. during the time of extreme summer and rainy season. In general, fungal spores exhibited peak concentration during early and late rainy season and also during the late winter.

3.1. Total airborne fungal spores in two sections (collected by the Burkard Sampler)

The spores of 'Aspergilli/Penicilli' dominated in both the sections. Other dominant spore types from two sections of the cattle shed were *Cladosporium*, *Nigrospora*, *Periconia*, *Choanephora*, *Corynespora*, *Curvularia*, *Drechslera*, *Memnoniella*, *Torula* and smut spores (Ustilaginales). Unidentified ascospores and basidiospores also contributed a large fraction to the total airborne spores. The average total spore concentration during the 2-year sampling was higher in the buffalo section (1220/m³) than the cow section (991/m³) and the difference was statistically significant ($p < 0.05$). Value of one-tail p was considered because the characteristic features of the buffalo section were more favorable for mold growth (see Section 2.1) and a higher airborne spore concentration was expected. Occurrence and seasonal duration of most fungal spore types demonstrated a general similarity in both years, but exceptions were noted in case of the spores of *Periconia*, *Leptosphaeria*, *Cladosporium*,

Choanephora and *Curvularia*. Comparing the 2 years' data it was observed that total average monthly spore concentration in the buffalo section ranged between 447 and 2206/m³ during the first sampling year and 233–2450/m³ in the second sampling year (Fig. 1). In the cow section of the cattle shed the range of total average monthly spore concentration was 253–2985/m³ during the first sampling year and 308–2544/m³ during the second sampling year (Fig. 1). Some noticeable differences in total spore concentrations between the 2 years were observed in the months of winter and late rainy season (Fig. 1), however, while comparing the overall total spore data of the 2 years in both sections, no statistically significant differences were observed in paired t -tests ($p > 0.05$).

3.2. Airborne culturable fungi in two sections (collected by the Andersen Sampler)

Among the recorded culturable molds, the dominant types originated from *Aspergillus niger*, *Aspergillus flavus*, *Alternaria alternata*, *Cladosporium cladosporioides*, *Absidia blakesleeana*, *Penicillium citrinum*, *Penicillium chrysogenum*, *Rhizopus nigricans* and *Syncephalastrum racemosum*. In contrast to the total spore data from the Burkard Sampler, the average total CFU concentration was lower (732/m³) at the buffalo section than the cow section (884/m³). This difference was, however, not statistically significant ($p > 0.05$). Occurrence and seasonal duration of the culturable fungal spores demonstrated a general similarity in both years, but exceptions were noted in case of the CFUs of *Aspergillus japonicus*, *Fusarium roseum*, *C. cladosporioides*, *P. chrysogenum*, *Absidia blakesleeana*, *R. nigricans* and *S. racemosum*. Regarding culturable molds in the buffalo section, CFU concentration varied between 153 and 1403 CFU/m³ in the first year and 166–2121 CFU/m³ in the second year (Fig. 2). The CFU concentration range in the cow section was 346–2202 CFU/m³ during the first year and

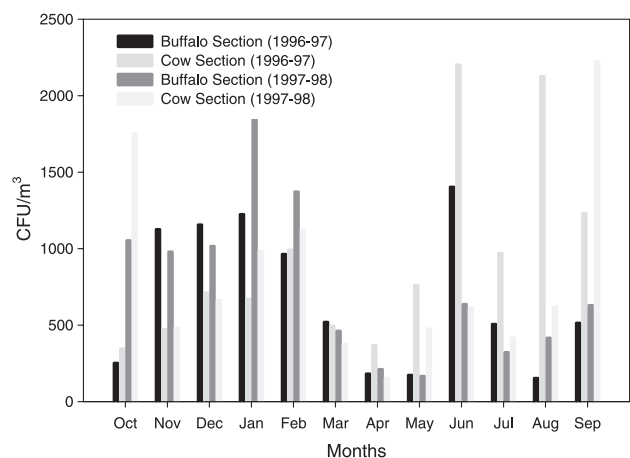


Fig. 2. Monthly averages of total viable spore concentrations (CFU/m³) in two sections of the cattle shed.

153–2225 CFU/m³ during the second year (Fig. 2). Although in the months of winter and late rainy season, some remarkable differences were present between the 2 years' data (Fig. 2), but similar to total airborne spores, while comparing the overall total CFU concentration between the 2 different years, no statistically significant differences were observed in paired *t*-tests ($p>0.05$).

3.3. Monthly variation patterns of airborne fungal spores in two sections, as recorded by the Burkard Sampler

The concentration of 'Aspergilli/Penicilli' was relatively higher at the buffalo section compared to all other fungal spores (1670/m³ during December of first year and 892/m³ during August of second year) followed by *Cladosporium* (1327/m³ in March of first and 1284/m³ during February of the second year). Other common spore types in this place were *Nigrospora*, *Periconia*, *Choanephora*, *Drechslera*, *Curvularia*, and *Memmoniella*. Average monthly concentrations of dominant airborne spore types from the 2-year

sampling by the Burkard Sampler in both sections of the cattle shed are presented in Table 1. The identification was done up to the genus level because species identification is not possible based on the spore morphology only. Except these dominant spores, all other identified sporadically recorded common spore types in both the sections were: *Cercospora*, *Chaetomium*, *Epicoccum*, *Thermomyces*, *Trichochonis*, *Phaeotrichochonis*, *Humicola*, *Tetraploa*, *Pithomyces*, *Melanosporea*, *Botryodiplodea*, *Pestalotia*, *Bispora*, *Sporidesmium* and *Bipolaris*. Few fungal spore types remained unidentified and placed in the group of 'unknown spores'.

3.4. Monthly variation patterns of airborne culturable fungi in two sections, as recorded by the Andersen Sampler

Regarding the culturable molds, highest CFU concentration was demonstrated by *A. flavus* (892 CFU/m³ during June of the first year and 796 CFU/m³ in January of the second year) followed by *C. cladosporioides* (555 and 768

Table 1

Monthly average concentration of dominant airborne fungal spores (including both culturable and non-culturable types) (No/m³) in two sections of the cattle shed during 2 sampling years

Fungal types	Sections	October	November	December	January	February	March	April	May	June	July	August	September	Overall contribution (%)
<i>Alternaria</i>	B	0	0	0	0	17	10	31	7	86	10	0	0	1.09
	C	0	0	0	2	4	9	0	10	52	5	6	0	0.74
<i>Aspergilli/Penicilli</i>	B	172	315	835	215	227	3	65	102	19	365	1001	608	26.82
	C	87	104	560	588	175	58	4	35	1146	558	706	388	37.09
<i>Choanephora</i>	B	0	0	0	2	3	9	26	358	22	17	2	0	2.99
	C	0	0	0	4	12	13	2	14	0	5	0	0	0.42
<i>Cladosporium</i>	B	30	107	101	740	803	978	147	0	27	0	0	0	20.03
	C	19	165	141	234	1112	1027	45	100	464	7	0	0	27.88
<i>Corynespora</i>	B	0	0	0	0	0	0	23	14	3	5	5	17	0.45
	C	0	0	0	10	16	11	19	6	0	0	0	0	0.52
<i>Curvularia</i>	B	22	11	4	5	11	18	7	10	7	7	0	0	0.69
	C	6	2	0	2	0	3	2	7	17	0	0	0	0.32
<i>Drechslera</i>	B	50	2	16	12	41	17	15	3	13	30	0	0	1.35
	C	26	20	14	16	27	23	7	10	0	20	10	9	1.53
<i>Memmoniella</i>	B	4	20	2	9	10	12	4	0	10	18	0	0	0.60
	C	4	4	2	4	12	26	4	5	17	2	0	0	0.67
<i>Nigrospora</i>	B	164	97	76	60	465	136	240	286	200	18	5	38	12.19
	C	140	94	58	53	72	95	148	39	223	32	35	57	8.80
<i>Periconia</i>	B	31	60	46	6	258	94	126	41	10	2	0	2	4.61
	C	11	63	22	55	68	40	43	56	70	6	0	0	3.65
<i>Torula</i>	B	54	6	6	4	20	8	22	3	0	10	12	5	1.02
	C	31	12	2	10	18	6	3	0	6	0	0	0	0.74
<i>Leptosphaeria</i>	B	0	5	0	12	21	13	11	8	2	0	7	4	0.56
	C	0	0	0	22	7	2	0	3	0	0	0	0	0.28
Unidentified ascospores	B	23	52	36	30	34	97	91	18	34	130	116	171	5.68
	C	36	21	13	17	31	35	251	59	19	15	38	92	5.27
<i>Ganoderma</i>	B	0	0	0	0	0	0	0	7	2	47	69	32	1.07
	C	7	0	0	0	0	0	0	5	3	23	20	21	0.66
Smut spores	B	7	12	29	18	21	44	13	19	0	0	9	13	1.26
	C	11	35	6	16	26	31	28	0	0	2	12	24	1.60
Unidentified basidiospores	B	0	25	0	2	12	8	97	18	29	37	93	141	3.15
	C	20	23	0	4	18	38	43	27	97	87	8	27	3.29

Abbreviations: B=buffalo section of the cattle shed; C=cow section of the cattle shed.

Table 2

Monthly average concentration of dominant airborne culturable fungi (CFU/m³) in two sections of the cattle shed during 2 sampling years

Fungal types	Sections	October	November	December	January	February	March	April	May	June	July	August	September	Overall contribution (%)
<i>Alternaria alternata</i>	B	0	0	0	15	4	5	10	2	0	0	4	3	0.48
	C	30	0	11	16	52	30	0	15	28	2	21	8	2.00
<i>Aspergillus niger</i>	B	21	326	11	7	90	91	25	32	246	47	28	65	11.26
	C	94	53	3	32	326	10	69	9	91	119	12	61	8.28
<i>Aspergillus flavus</i>	B	320	17	93	613	600	43	22	10	600	69	18	71	28.20
	C	591	68	182	116	35	49	12	62	487	221	398	326	24.00
<i>Aspergillus fumigatus</i>	B	22	4	7	0	0	10	0	26	8	23	0	134	2.66
	C	0	6	1	1	0	38	34	13	32	12	38	412	5.53
<i>Aspergillus japonicus</i>	B	1	0	1	2	6	6	2	33	73	39	4	22	2.15
	C	1	0	0	5	9	17	1	34	316	26	6	7	3.97
<i>Aspergillus ochraceous</i>	B	0	0	2	3	0	0	0	2	12	2	0	10	0.35
	C	0	0	2	3	0	1	4	2	3	0	0	0	0.14
<i>Caldosporium oxysporum</i>	B	0	0	4	41	8	0	0	0	0	0	0	0	0.60
	C	0	0	6	7	20	8	2	0	0	0	0	0	0.40
<i>Caldosporium cladosporioides</i>	B	0	9	325	662	136	43	29	0	0	0	0	0	13.71
	C	0	19	138	410	214	24	1	0	0	0	0	0	7.59
<i>Fusarium roseum</i>	B	15	126	16	13	43	14	8	0	0	0	0	0	2.67
	C	6	12	10	10	20	17	10	4	18	0	0	0	1.00
<i>Penicillium chrysogenum</i>	B	0	0	167	7	53	14	0	0	0	22	6	10	3.17
	C	0	0	15	16	5	6	11	4	306	22	34	49	4.41
<i>Penicillium citrinum</i>	B	0	329	229	122	34	78	4	0	0	23	21	24	9.84
	C	80	157	92	14	7	24	63	56	58	53	22	31	6.19
<i>Absidia blakesleeana</i>	B	0	12	38	11	18	24	24	0	8	0	0	0	1.53
	C	3	15	22	4	7	8	20	0	10	0	0	1	0.84
<i>Mucor racemosus</i>	B	6	14	21	6	2	0	0	0	4	0	0	0	0.60
	C	2	2	9	11	1	0	0	0	1	19	26	23	0.88
<i>Rhizopus nigricans</i>	B	4	5	9	23	29	0	2	0	18	51	31	14	2.11
	C	0	2	12	8	0	1	2	0	0	0	0	0	0.23
<i>Rhizopus stolonifer</i>	B	0	0	0	4	15	2	2	0	6	8	10	0	0.53
	C	0	2	12	8	0	1	2	0	0	0	0	0	0.23
<i>Syncephalastrum racemosum</i>	B	6	0	0	0	0	0	0	0	0	10	10	18	0.50
	C	3	6	1	7	9	4	2	3	3	0	4	15	0.53

Abbreviations: B=buffalo section of the cattle shed; C=cow section of the cattle shed.

CFU/m³ during January of first and second years, respectively). The next dominance was showed by *A. niger* and *P. citrinum*. With regard to culturable molds in cow section, maximum CFU concentration was demonstrated by *A. flavus* (868 CFU/m³ during June of first and 1165 CFU/m³ during October of the second year) followed by *C. cladosporioides* (357 and 463 CFU/m³ during January of first and second years, respectively). CFUs of *P. citrinum* and *A. niger* were also recorded in a higher concentration. Average monthly airborne CFU concentrations (CFU/m³) of the predominant culturable molds (identified up to species level) from the 2-year sampling at both sections are presented in Table 2. All other sporadically recorded fungal colonies were: *Aspergillus parasiticus*, *Aspergillus nidulans*, *Aspergillus candidus*, *Aspergillus clavatus*, *Aspergillus glaucus*, *Aspergillus terreus*, *Aspergillus versicolor*, *Penicillium oxalicum*, *Penicillium rugulosum*, *Cladosporium herbarum*, *Paecilomyces lilacinus*, *Paecilomyces variotti*, *Trichoderma harzianum*, *Drechslera coisis*, *Curvularia pallescens*, *Chaetomium globosum*, *Saccharomyces cerevisiae*, *Gliocladium roseum* and *Epicoecum nigrum*. Several fungal colonies did not

sporulate after incubation for a long time and remained unidentified. These colonies were placed in the 'non-sporulating colonies' group.

Table 3

Average temperature and relative humidity recorded in two sections of the cattle shed and average monthly rainfall of the locality from 2 sampling years

Months	Temperature (°C)		Relative humidity (%)		Rainfall (mm)
	Cow section	Buffalo section	Cow section	Buffalo section	
October	28.8	27.5	58	61	5.3
November	26.3	25.0	61	68	0.3
December	21.0	19.8	46	55	0.1
January	22.1	20.3	49	54	1.2
February	26.8	25.3	58	64	0.6
March	30.8	30.4	45	46	1.9
April	32.4	32.2	64	67	2.7
May	35.2	34.4	76	82	5.5
June	33.8	33.8	70	72	8.3
July	33.1	32.6	85	87	20.4
August	31.1	30.2	82	83	17.5
September	29.9	29.6	86	88	21.3

3.5. Record of meteorological parameters

Temperature (°C) and relative humidity (%) were recorded in each spot during the sampling. But data on the daily average rainfall (mm) were collected from the nearest meteorological office at Alipore. Monthly averages of temperature and relative humidity recorded during sampling and monthly averages of rainfall collectively from both the years are presented in Table 3.

4. Discussion

The information on the airborne fungal flora of the cattle shed from this 2-year study will be useful while dealing with the occupational health problems like respiratory allergy and incidence of pulmonary function impairment symptoms among the workers of cattle sheds and dairy farmers. There are few reports on the airborne fungi and fungal aeroallergens in dairy barns of USA (Kullman et al., 1998; Campbell et al., 1989), organic dusts including airborne fungi in dairy barns of Canada (Duchaine et al., 1999), airborne fungi in calf houses of Denmark (Blom et al., 1984) and airborne fungi in Finnish cow barns (Hanhela et al., 1995). However, except the Danish study, all other studies were performed for a short duration of time, and except the Finnish study, all other studies were restricted to airborne viable fungi only. It appears that there is no report on both viable and non-viable airborne fungi in cattle sheds or dairy farms based on long-term air-sampling experiments. The present study is anticipated to fill this knowledge gap.

The present study showed that, in both sections of the cattle shed, more than 50% of the total fungal spores were contributed by the Aspergilli/Penicilli group, *Cladosporium* and *Nigrospora* (Table 1). Higher concentration of Aspergilli/Penicilli and *Cladosporium* has been reported from many other indoor environments of the same study area (Adhikari, 2000), like a bakery, a poultry shed and a vegetable market, but higher concentration of *Nigrospora* was uniquely observed in the cattle shed. The feed for livestock, especially the stacks of straw might serve as a local source of *Nigrospora* spores in the cattle shed. With respect to the culturable molds, *A. flavus*, *A. niger*, *C. cladosporioides* and *P. citrinum* collectively contributed more than 50% of the total CFUs (Table 2). Thus, the use of the antigenic extracts of these four fungi and also the extract of *Nigrospora* spores can be recommended for the primary skin-prick testing of dairy farmers of this area while evaluating the mold allergen sensitivity.

Wet and humid conditions induce decomposition of raw organic materials in cattle sheds, which provide suitable condition for growth of fungi consequently increasing the airborne spore load. The winter season in India belongs to the months between October and February, summer from March to June and rainy season (monsoon) from July to September. Seasonal variations in the total spore concen-

tration and CFU concentration were observed during this study. Higher total spore and CFU concentration levels were found in the months of the winter and late rainy season compared to the months of the summer. This observation showed some contrasting result compared to a year-round airborne fungal study at the calf houses in Denmark (Blom et al., 1984), which showed lower fungal count in the winter. The large temperature difference during the winter in India and Denmark might be the responsible factor. With respect to the individual fungal types, seasonal variations in some fungal spores like *Cladosporium*, *Alternaria*, *Curvularia*, *Drechslera*, *Trichochonis*, *Phaeotrichochonis*, *Ganoderma* and CFUs of *C. cladosporioides*, *A. alternata*, *P. lilacinus* and *F. roseum* were noted in both sections of the cattle shed.

More fungal growth substrates were observed at the buffalo section of the cattle shed compared to the cow section. Ventilation was also inadequate there. A statistically significant higher spore concentration at the buffalo section can be justified because of these factors. No such significant difference was observed in case of culturable fungi. The only possible explanation for this observation is the presence of some specific growth substrates at the buffalo section that supported the growth of fungi, which were not culturable in Sabourauds Rose Bengal Agar media. Although some differences were observed between the 2 years with respect to the CFU and spore concentrations of individual fungal types and average monthly concentrations, but no statistically significant difference was observed when the total overall yearly concentrations were considered. The possible reason might be the insignificant changes of environmental factors as well as the fungal growth substrates at the cattle shed during the 2 sampling years.

It is an established fact that temperature and relative humidity are two important factors for fungal spore generation, release and dispersal; particularly in indoor environments (Herrero and Zaldivar, 1997; Larsen and Frisvad, 1994). Higher temperature (°C) and relative humidity (%) in the months of rainy season (Table 3) and associated higher concentration of culturable fungal spores in the cattle shed are supporting the observations of earlier researchers.

Outdoor airborne fungi sometimes influence the levels of airborne concentration in indoors (Burge, 1990). In the present study, no control outdoor sampling was performed exactly outside the cattle shed. However, the outdoor fungal spore data measured at the same time using the same samplers in a sampling station 1 km away from the cattle shed is available, which has been published elsewhere (Adhikari, 2000). The average total fungal spore concentration at the outdoor station was 600/m³ in the first year and 495/m³ in the second year. The range of total spore concentration at the outdoor station was 248–1176/m³ in the first year and 156–1215/m³ in the second year. Regarding the culturable fungi, the average total CFU concentration at the outdoor station was 454 CFU/m³ in the first year and 544/m³ in the second year. The range of total CFU

concentration at the outdoor station was 145–1796 and 215–1592 CFU/m³ in the first and the second years, respectively. While comparing the indoor and outdoor fungi for a whole year, an earlier Finnish study (Pellikka et al., 1985) reported almost same concentration levels of viable airborne fungi in urban indoor home environments (150–170 CFU/m³) and urban outdoor air (175 CFU/m³). However, when the total outdoor spore data and CFU data were compared to the similar data from the two sections of the cattle shed, an obvious higher concentration was noted in both cattle shed sections. This observation can be attributed to the presence of substantial fungal growth substrates and the higher levels of relative humidity inside the cattle shed unlike to indoor home environments.

While comparing the investigated cattle shed to the dairy barns, cow barns and calf houses of the other parts of the world with respect to the airborne culturable and total fungal spores, lower concentration levels were observed in the present study. Kullman et al. (1998) reported a mean concentration of 10⁴ CFU/m³ for the airborne viable molds at the dairy barns in central Wisconsin, USA. Hanhela et al. (1995) found the concentrations of 10²–10⁷ CFU/m³ for viable fungi and 10⁵–10⁷/m³ for total fungal spores in Finnish cow barns. Duchaine et al. (1999) reported about 10⁶ CFU/m³ levels of viable airborne fungi from the dairy farms of Quebec, Canada and Blom et al. (1984) found a mean concentration level of 10³–10⁵ CFU/m³ in Danish calf houses. In our study, the overall concentration range for the total fungal spores was 2.3×10^2 – 2.9×10^3 /m³ and for the airborne viable fungi, the range was 1.6×10^2 – 2.2×10^3 CFU/m³. The different climatic conditions, fungal types, and fungal growth substrates in different countries might be the cause for this difference. The total and viable fungal spore concentration levels, however, might be enough for evoking allergic symptoms in cattle shed workers. While evaluating the mold allergy in other indoor environments, Reponen et al. (1992) and Gravesen (1979) indicated that a level of 500 CFU/m³ might be considered as unacceptable and 3000 CFU/m³ of *Cladosporium* sp. and 100 CFU/m³ of *Alternaria* sp. may function as a threshold limit for evoking allergic symptoms.

It has been reported that a substantial amount of toxins may be absorbed from the inhaled fungal spores (Flannigan et al., 1991). Some recorded toxic fungi in this cattle shed could have contributed toxins to stored straws and fodders for cows and sometimes to the milk. Particularly, the >25% contribution of *A. flavus* (Table 2), a potent source of the carcinogenic aflatoxin, might be a matter of concern for the cattle shed workers and the people used to visit this place. To prevent the associated health hazards, the owners of cattle sheds should take necessary precautions against fungal growth, may be by using Thimerosol (0.02%), Formaldehyde (0.2–0.5%), or other chlorine bleaches (Standard and Kaufman, 1982). The ventilation systems could be improved for reducing the relative humidity levels. Straws should be adequately dried before feeding cattle and fresh

uncontaminated livestock foods should be used. Higher concentrations of the spores of *Cladosporium*, *Alternaria* and *Penicillium* were recorded in both sections, which could contaminate the dairy products prepared from the milk produced in the cattle shed (Ranjan and Sinha, 1991).

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References

- Adhikari, A. Investigations on aero-mycology in relation to allergy in some selected semi-rural places of West Bengal, India. 2000. PhD Dissertation, Jadavpur University, Calcutta, India.
- Andersen AA. New sampler for collection, sizing and enumeration of viable airborne particles. *J Bacteriol* 1958;76:471–84.
- Blom JY, Madsen EB, Krogh HV, Wolstrum J. Numbers of airborne bacteria and fungi in calf houses. *Nord Vet Med* 1984;36:215–20.
- Burge H. Bioaerosols: prevalence and health effects in the indoor environment. *J Allergy Clin Immunol* 1990;86:687–701.
- Campbell AR, Swanson MC, Fernandez-Caldas E, Reed CE, May JJ, Pratt DS. Aeroallergens in dairy barns near Cooperstown, New York and Rochester, Minnesota. *Am Rev Respir Dis* 1989;140:317–20.
- Dalphin JC, Dubiez A, Monnet E, Gora D, Westeel V, Pernet D, et al. Prevalence of asthma and respiratory symptoms in dairy farmers in the French province of the Doubs. *Am J Respir Crit Care Med* 1998a;158:1493–8.
- Dalphin JC, Maheu MF, Dussaucy A, Pernet D, Polio JC, Dubiez A, et al. Six year longitudinal study of respiratory function in dairy farmers in the Doubs province. *Eur Respir J* 1998b;11:1287–93.
- Duchaine C, Mériaux A, Brochu G, Cormier Y. Airborne microflora in Quebec dairy farms: lack of effect of bacterial hay preservatives. *AIHAJ* 1999;60:89–95.
- Ebner MR, Haselwandter K. Indoor and outdoor incidence of airborne fungal allergens at low and high-altitude alpine environments. *Mycol Res* 1992;96:117–24.
- Flannigan B, McCabe EM, McGarry F. Allergenic and toxigenic microorganisms in houses. *J Appl Bacteriol* 1991;61S–73S [Symposium Supplement].
- Gravesen S. Fungi as a cause of allergic diseases. *Allergy* 1979;34:135–64.
- Hanhela R, Louhelainen K, Pasanen AL. Prevalence of microfungi in Finnish cow barns and some aspects of the occurrence of *Wallemia sebi* and *Fusaria*. *Scand J Work, Environ & Health* 1995;21:223–8.
- 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;36:180–4.
- Kullman GJ, Thorne PS, Waldron PF, Marx JJ, Ault B, Lewis DM, et al. Organic dust exposures from work in dairy barns. *AIHAJ* 1998;59:403–13.

- Lacey J. The Aerobiology of Conidial fungi. In: Cole GT, Kendrick B, editors. *Biology of conidial fungi*. New York: Academic Press; 1981. p. 373–416.
- Lacey J, Crook B. Fungal and actinomycete spores as pollutants of the work place and occupational allergens. *Ann Occup Hyg* 1988;32: 515–33.
- Larsen TO, Frisvad JC. Production of volatiles and mycotoxins in conidia of common *Penicillia* and *Aspergilli*. In: Samson RA, Flannigan B, editors. *Health implications of fungi in indoor environments*. Amsterdam: Elsevier; 1994. p. 251–79.
- Lehrer SB, Aukrust L, Salvaggio JE. Respiratory allergy induced by fungi. *Clin Chest Med* 1983;4:23–41.
- Luo W. Deposition of large particles in the nose and mouth. *Grana* 1991; 30:79–81.
- Lynch JM, Poole NJ. Aerial dispersal and the development of microbial communities. In: Lynch JM, Poole NJ, editors. *Microbial ecology: a conceptual approach*. New York: Wiley; 1979. p. 140–70.
- Pellikka M, Jantunen MJ, Kalliokoski P, Pitkänen E. Ventilation and bio-aerosols. Paper presented at the first international symposium on ventilation for contaminant control; 1985. Toronto, Canada.
- Pepys J. Hypersensitivity disease of the lung due to fungi and organic dusts. Basel: S. Karger; 1965.
- Ranjan KS, Sinha AK. Occurrence of mycotoxigenic fungi and mycotoxins in animal feed from Bihar. *Ind J Sci Food Agric* 1991;56(1):39–47.
- Reponen T, Nevalainen A, Jantunen MJ, Pellikka M, Kalliokoski P. Normal range criteria for indoor air bacteria and fungal spores in a subarctic climate. *Indoor Air* 1992;2:26–31.
- Singh AB, Singh A. Indoor airborne fungi as important occupational sensitizers in poultry workers. *Indoor Built Environ* 1996;5:138–47.
- Standard PG, Kaufman L. Safety considerations in handling exoantigen extracts from pathogenic fungi. *J Clin Microbiol* 1982;15:663–7.
- Stetzenbach LD. Microorganisms and indoor air quality. *Clin Microbiol Newsl* 1998;20:157–61.
- Takahasi T. Airborne fungal colony-forming units in outdoor and indoor environments in Yokohama, Japan. *Mycopathologia* 1997;139:23–33.
- Vittal BPR, Glory A. Airborne fungus spores of a library in India. *Grana* 1985;24:129–232.
- Westeel V, Julien S, De Champs C, Polio JC, Mauny F, Gibey R, et al. Relationships of immunoglobulins E and G sensitization to respiratory function in dairy farmers. *Eur Respir J* 2000;16:886–92.